NIEHS AIDS Therapeutics
Toxicity Report
Number 7

**NIEHS Technical Report
on the Reproductive, Developmental,
and General Toxicity Study of**

3'-Azido-3'-deoxythymidine (AZT) and Pyrazinamide Combinations

(CAS Nos. 30516-87-1 and 98-96-4)

**Administered by Gavage
to Swiss CD-1® Mice**

**NIH Publication 02-4408
April 2002**

**U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health**

FOREWORD

Infection with human immunodeficiency virus (HIV) causes immunosuppression and leads to acquired immunodeficiency syndrome (AIDS) with a broad spectrum of opportunistic infections. Prophylaxis and treatment of AIDS are generally combination therapies of antiretroviral agents with antimicrobial drugs specific for the opportunistic infections. The National Institute of Environmental Health Sciences (NIEHS), under the AIDS research program, is evaluating AIDS therapeutics for reproductive, developmental, and general toxicity in rodents. These evaluations may include single therapeutic agents or combination therapies when the toxic potential of these agents in animal models is not available or is incomplete.

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CONTRIBUTORS

This report on the reproductive, developmental, and general toxicity study of 3'-azido-3'-deoxythymidine (AZT) and pyrazinamide combinations is based primarily on a study that began in January 1995 and ended in March 1995 at Southern Research Institute, Birmingham, AL.

National Institute of Environmental Health Sciences

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PEER REVIEW

The draft report on the reproductive, developmental, and general toxicity study of 3'-azido-3'-deoxythymidine (AZT) and pyrazinamide combinations was evaluated by the following reviewers. These reviewers served as independent scientists, not as representatives of any institution, company, or government agency. In this capacity, reviewers determine if the design and conditions of the study are appropriate and ensure that this reproductive, developmental, and general toxicity study report presents the experimental results and conclusions fully and clearly. The comments of the reviewers were reviewed prior to finalization of this document. Changes were made such that the concerns of the reviewers were addressed to the extent possible.

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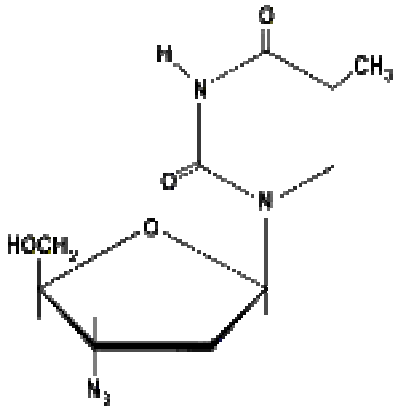
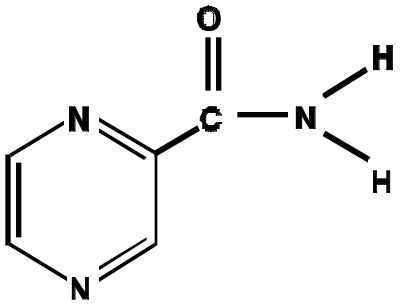
CONTENTS

ABSTRACT

3'-Azido-3'-deoxythymidine (AZT) and Pyrazinamide Combinations



AZT
Molecular Formula: $C_{10}H_{13}N_5O_4$
Molecular Weight: 267.24
CAS No.: 30516-87-1



Pyrazinamide
Molecular Formula: $C_5H_5N_3O$
Molecular Weight: 123.12
CAS No.: 98-96-4

Male and female Swiss CD-1® mice were dosed orally with AZT alone (200 or 400 mg/kg per day), pyrazinamide alone (300, 1,500, or 3,000 mg/kg per day), or combinations of AZT and pyrazinamide. The doses of AZT were equivalent to 2 and 4 times the therapeutic dose in humans, based on body surface area, and the doses of pyrazinamide were 2, 10, and 20 times the therapeutic dose for experimental tuberculosis in mice (Freireich *et al.,* 1966; Grosset *et al.,* 1992; *PDR*, 1999). Male mice (10 per group) were dosed from day 5 until the day prior to sacrifice on day 25 or 26. Females were divided into two groups designated female-A mice and female-B mice. The female-A mice (20 per group) were dosed from day 0 to the day of sacrifice. They were cohabited with treated males on days 9 to 13 to test for effects of treatment on mating behavior, fertilization, and implantation, and caesarean sections were performed on days 28 to 32. Female-B mice (20 per group) were cohabited with untreated males on days 0 to 4. Sperm-positive female-B mice were dosed during organogenesis on days 6 to 15 of presumed gestation and sacrificed on day 4 of lactation. A summary of the most significant toxicological parameters is presented in Table 1.

TABLE 1
Summary of Significant Treatment-Related Toxicological Parameters in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Parameter and Treatment Regimen	Male Mice	Female-A Mice	Female-B Mice
Hematology			
AZT Alone	Mild anemia Slight declines in erythrocyte counts and hemoglobin and hematocrit values Slight elevations in mean cell volumes and mean cell hemoglobin values Slight increase in platelet counts	Mild anemia Elevations in mean cell volumes and mean cell hemoglobin values Slight increase in platelet counts Mild leukopenia Mild neutropenia	Slight elevations in mean cell volumes
Pyrazinamide Alone	Slight increase in platelet counts	No significant alterations	No significant alterations
AZT plus Pyrazinamide	Mild to moderate anemia Elevations in mean cell volumes and mean cell hemoglobin values in lower-dose combination groups Slight reticulocytopenia Thrombocytosis Slight leukopenia Slight neutropenia Slight lymphopenia	Moderate anemia Elevations in mean cell volumes and mean cell hemoglobin values Thrombocytosis Leukopenia Neutropenia Lymphopenia	Slight elevations in mean cell volumes and mean cell hemoglobin values
Body and Organ Weights			
AZT Alone	No effect	Decreases in final body weight and gravid uterine weight No effect on corrected body weight Slight decreases in absolute and relative liver weights	No effect
Pyrazinamide Alone	Slight increase in body weight Slight increase in relative liver weight Slight decrease in relative epididymal weight	No effect on final body weight or corrected body weight Decrease in gravid uterine weight Increases in absolute and relative liver weights	No effect
AZT plus Pyrazinamide	Slight increase in body weight Increases in liver weights	Decreases in final body weight and gravid uterine weight No effect on corrected body weight No treatment-related effect on liver weights	Slight decrease in gestational body weight in higher-dose combination groups

TABLE 1
Summary of Significant Treatment-Related Toxicological Parameters in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Parameter and Treatment Regimen	Male Mice	Female-A Mice	Female-B Mice
Histopathology[a]			
AZT Alone	Slight hepatocellular glycogen depletion Slight hematopoietic cell proliferation in spleen	Slight hepatocellular glycogen depletion	Not evaluated
Pyrazinamide Alone	Hepatocellular glycogen depletion Slight hematopoietic cell proliferation in spleen	Hepatocellular glycogen depletion	Not evaluated
AZT plus Pyrazinamide	Hepatocellular glycogen depletion Bone marrow depletion Hematopoietic cell proliferation in spleen Atrophy of splenic red pulp	Hepatocellular glycogen depletion	Not evaluated
Reproductive/Developmental			
AZT Alone	Slight increase in left caudal weight Slight decrease in epididymal sperm motility	Decreases in live litter size and fetal weight per litter Increase in resorptions	Slight increase in number of pups dying during lactation period Slight decrease in live litter size and pup body weight per litter
Pyrazinamide Alone	Slight decrease in left testis weight	Slight increase in resorptions Slight decrease in fetal weight per litter	Increases in duration of gestation and number of pups dying during lactation period Slight decrease in live litter size and pup body weight per litter
AZT plus Pyrazinamide	Slight increase in the number of spermatid heads per gram of testis	Decreases in pregnancy rate, live litter size, and fetal weight per litter Increase in resorptions	Increases in duration of gestation, percent of dams with stillborn pups, and number of pups dying during lactation period Decreases in number of delivered litters, litter size, liveborn pups per litter, and pup body weight per litter

[a] Only liver was evaluated in female-A mice. Histopathology was not evaluated in female-B mice.

Administration of AZT alone to male mice resulted in a mild dose-related anemia accompanied histopathologically by hematopoietic cell proliferation in the spleen. Administration of pyrazinamide alone to male mice did not produce significant alterations in hematology parameters, and histopathological alterations were limited to minor degrees of hepatocellular glycogen depletion and hematopoietic cell proliferation in the spleen. Administration of combinations of AZT and pyrazinamide to male mice resulted in exacerbation of the anemia produced by AZT alone. Combination therapy in male mice also resulted in diminished leukocyte, neutrophil, and lymphocyte counts and a treatment-related thrombocytosis. Significant histopathological alterations consisted of bone marrow depletion, hematopoietic cell proliferation in the spleen, atrophy of the splenic red pulp, and hepatocellular glycogen depletion.

Administration of AZT alone to female-A mice resulted in a mild dose-related anemia. Biologically significant alterations were not evident in hematology parameters of female-A mice treated with pyrazinamide alone and histopathological changes were limited to hepatocellular glycogen depletion. Administration of combinations of AZT and pyrazinamide to female-A mice resulted in exacerbation of the anemia induced by AZT alone. Female-A mice receiving combination therapy also developed a treatment-related leukopenia, with declines in granulocytes and mononuclear cells. A biologically significant thrombocytosis occurred in female-A mice treated with the highest combination of both drugs. Hepatocellular glycogen depletion was prominent in female-A mice receiving combination therapy and coincided with significant elevations in relative liver weights. The only significant clinical sign detected in female-A mice was pallor documented in a few of the more anemic animals.

Biologically significant declines in body weight were only evident in female mice. Terminal body weights and gravid uterine weights were diminished in female-A mice treated with AZT alone and AZT plus pyrazinamide. Corrected body weights, however, were similar to those of the controls. A slight decline in gestational body weight occurred in female-B mice treated with the highest combination of both drugs. Significant alterations in mortality, clinical chemistry parameters, and necropsy findings were not evident in any treated female groups. Biologically significant alterations in hematology parameters did not occur in any of the female-B treatment groups.

Administration of AZT alone resulted in reproductive toxicity manifested by an increase in the number of resorptions, diminished litter size, and diminished fetal weights per litter. Pyrazinamide alone resulted in a mild increase in the

number of resorptions, diminished fetal body weights per litter, and a mild increase in the duration of gestation. Combination therapy resulted in alterations in the above reproductive parameters of far greater magnitude than occurred with either compound alone. Other changes evident subsequent to combination therapy consisted of a decrease in the number of pregnant females that delivered litters, diminished litter size, an increase in the number of stillborn pups, and a decrease in the number of liveborn pups per litter. Combination therapy also resulted in diminished live litter size and an increase in the number of pup deaths during the lactation period. Significant numbers of gross external alterations did not occur following administration of either compound alone or in combination.

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a lethal multi-system disease that has become a major public health problem since its recognition in 1981 (Gottlieb *et al.*, 1981; Masur *et al.*, 1981; Siegle *et al.*, 1981). The etiological agent of AIDS is a retrovirus that is now referred to as the human immunodeficiency virus (HIV) (Coffin, 1986). To date, the most effective single agent in the treatment of HIV has been the first dideoxynucleoside analogue used in clinical trials, zidovudine (3'-azido-3'-deoxythymidine, AZT, Retrovir, azidothymidine, compound S, BW A509U, CAS No. 30516-87-1), commonly referred to as AZT (Vince *et al.*, 1988; Amin, 1989).

AZT therapy produces numerous beneficial effects in AIDS patients, including decreases in morbidity and increases in life span (Amin, 1989; Jeffries, 1989). The most important adverse effects of AZT are anemia and granulocytopenia, which are believed to reflect bone marrow toxicity (Richman, 1988; Amin, 1989). Two types of anemia may occur with AZT therapy: macrocytic megaloblastic anemia and normocytic normochromic anemia.

Several subacute and subchronic rodent toxicity studies have demonstrated that the primary toxicity of AZT is myelosuppression. Male Swiss CD-1® mice were dosed daily by gavage with AZT doses of 100, 250, 500, or 1,000 mg/kg for 30 days (Mansuri *et al.*, 1990). No mortality or body weight effects were evident from AZT treatment. Erythropenia and increased mean cell volume were observed at all doses, and anemia was observed at the 1,000 mg/kg dose. Pathologic findings in the AZT-treated mice were consistent with the hematological results and included lymphoid depletion, reticuloendothelial hyperplasia in the spleen and thymus, and bone marrow hypocellularity.

In a 14-week study (NTP, 1999), B6C3F$_1$ mice were treated with 0, 25, 50, 100, 400, or 1,000 mg of AZT/kg administered by gavage. On day 5, statistically significant dose-related decreases were observed in reticulocyte counts in males and females. Dose-related anemia was evident on days 23 and 93. To evaluate the ability of treated animals to reverse any compound-related effects when treatment is stopped, additional groups were administered doses of 0, 50, 400, or 1,000 mg of AZT/kg twice daily for approximately 92 days and then held without additional treatment for 29 days. Improvement of hematology parameters indicated recovery of the bone marrow after treatment stopped. An apparently nontoxic, treatment-related clinical observation in AZT-treated B6C3F$_1$ mice was a darkening of the skin on the tail, feet, and/or muzzle (Rao *et al.*, 1998).

Oral bioavailability of AZT was determined in female $B6C3F_1$ mice by comparison of the area under the curve obtained from an oral dose to that of an intravenous dose at the same concentration (Trang *et al*., 1993). Bioavailability was found to be 0.86, 0.78, and 0.97 for the 15, 30, and 60 mg/kg oral doses. The mean elimination half-life values ranged from 17.3 to 19.9 minutes for the three intravenous doses and from 16.5 to 21.9 minutes for the three oral doses. Based on these results, the internal dose of AZT was linear and dose-proportional over the oral dose concentration range.

Standard teratology tests of AZT have been performed in rats and rabbits (Ayers, 1988). Rats were dosed orally with 125 to 500 mg/kg on gestation days 6 to 15. No fetal toxicity or teratogenicity was found. Fetal AZT concentrations 30 minutes post-dosing were 61 μg/g or 76 times the antiviral ID_{50}. Rabbits were dosed orally at 125 to 500 mg/kg on gestation days 6 to 18, and no fetal toxicity or teratogenicity was found. Fetal AZT concentrations 30 minutes post-dosing were 40.2 μg/g or 50 times the antiviral ID_{50}.

Female Wistar rats were dosed orally three times with 100 mg/kg AZT at 5-hour intervals on gestation day 10 for a total dose of 300 mg/kg (Greene *et al*., 1990). No adverse effects on maternal weight gain, food consumption, hematological parameters, and growth or survival of offspring were observed. AZT concentration measurements 30 minutes after the last dose were 62.6 μg/mL in maternal plasma and 21.1 μg/g in fetal tissue.

Studies in mice concluded that AZT has a direct toxic effect on the developing mouse embryo (Toltzis *et al*., 1991). Female C_3H/He mice were exposed to 0, 0.25, 0.5, or 2.5 mg/mL of AZT in the drinking water for 8 weeks during mating and throughout gestation. All AZT groups had fewer pregnant mice per group, fewer pups per litter, and increased resorptions per mouse. Dose-related embryolethality was observed.

Since AIDS is a disease of immune suppression, the majority of AIDS patients actually die from characteristic opportunistic infections (Hardy, 1991; Harkins and Herriot, 1992). As a result, the treatment of AIDS is increasingly one of combination therapy of anti-AIDS drugs and anti-infective drugs (Goldschmidt and Dong, 1992). Tuberculosis (TB) is one of the opportunistic diseases leading to mortality in AIDS patients (Nolan, 1992), and the spread of the HIV virus is one of the factors contributing to the resurgence of TB and resistant strains of the microorganisms that cause TB (Humma, 1996). The prevalence of TB in HIV-infected individuals is high and the largest increases in TB nationally and internationally have occurred in urban areas that have a high percentage of HIV-infected individuals (Blanchard, 1996).

AIDS patients with TB receive combination therapy with AZT and antituberculosis drugs (CDC, 1998a). Treatment for TB involves combination therapy with multiple antibacterial agents in order to eliminate the strains of organisms

inducing the disease, including those resistant to isoniazid, the primary drug used in treating TB. The standard regimen is isoniazid (300 mg/day), rifampicin (600 mg/day; 450 mg/day for persons weighing less than 50 kg), and pyrazinamide (20 to 30 mg/kg per day) for the first 2 months of treatment. Isoniazid and rifampicin are continued for another 7 months, for a total therapy duration of 9 months (CDC, 1987; Barnes *et al*., 1991). For patients intolerant of rifampicins, combinations of isoniazid, streptomycin, ethambutol, and pyrazinamide are used, and rifabutin is substituted for rifampicin in patients receiving antiretroviral protease inhibitors (CDC, 1998a). The inclusion of pyrazinamide in treatment regimens has made possible the shortening of therapy to 6 months (East and Central African/British Medical Research Council, 1986).

Pyrazinamide is an analogue of nicotinamide and is thought to be a pro-drug that requires deamidation by an endogenous mycobacterial enzyme, pyrazinamidase, to form pyrazinoic acid (Barry, 1997). Pyrazinoic acid is thought to be a toxic metabolite, but the precise cellular functions inhibited by this molecule are not known (Blanchard, 1996; Barry, 1997). Pyrazinamide appears to be effective *in vivo* against semidormant bacilli in acidic intracellular environments such as macrophages (Blanchard, 1996).

Pyrazinamide is well absorbed from the gastrointestinal tract, and it is widely distributed throughout the body. The oral administration of 1 gram produces plasma concentrations of about 45 μg/mL at 2 hours and 10 μg/mL at 15 hours. The drug is excreted primarily by renal glomerular filtration; urinary concentrations are 50 to 100 μg/mL for several hours after a single dose. Pyrazinamide is hydrolyzed to pyrazinoic acid and subsequently hydroxylated to 5-hydroxypyrazinoic acid, the major excretory product (Weiner and Tinker, 1972). Grossett *et al*. (1992) reported that 150 mg/kg pyrazinamide (considered to be a therapeutic dose) administered by gavage to female Swiss CD-1® mice resulted in serum concentrations similar to those of humans receiving pyrazinamide therapy.

Injury to the liver is the most common and serious side effect of pyrazinamide. When a dose of 3 grams per day (40 to 50 mg/kg body weight) is orally administered, signs and symptoms of hepatic disease appear in about 15% of patients, with jaundice in 2% to 3% and death due to hepatic necrosis in rare instances (Mandel and Sande, 1990). Mild and transient elevations of plasma alanine and aspartate aminotransferases are the earliest abnormalities produced by the drug and occur approximately 2 weeks after initiation of therapy (Ramakrishnan *et al*., 1968). Baron and Bell (1974) have pointed out that such transient asymptomatic increases in the serum hepatic enzyme concentrations are also common during the early weeks of anti-TB chemotherapy with other drugs, and these usually return to normal without interrupting or altering the treatment and so are not clinically important.

In a review of the hepatotoxicity of antitubercular treatments in humans, pyrazinamide was referred to as a major hepatotoxin (Durand *et al*., 1996); however, the molecular mechanism for the toxicity of pyrazinamide has not been established. The author described two patterns of fulminant liver injury in patients receiving combinations of isoniazid, rifampicin, and pyrazinamide. The first pattern consists of an increase in serum transaminase activity within 15 days

after initiation of treatment. This pattern is believed to be rifampicin-induced isoniazid hepatotoxicity and has a good prognosis. The second pattern consists of increased serum transaminase activity more

than 1 month after start of treatment. This pattern may be related to pyrazinamide hepatotoxicity and has a poor prognosis. The frequency of hepatotoxicity with combination antitubercular treatments rose from approximately 0.5% in patients not receiving pyrazinamide to approximately 2% in patients receiving antitubercular treatments that included pyrazinamide.

Hepatotoxicity and nephrotoxicity occurred in male Wistar rats following the administration of 2.5 g/kg of pyrazinamide by gavage in 10% gum arabic (Zitkova *et al*., 1983). Bederka *et al*. (1975) have reported an LD_{25} for pyrazinamide in young adult female Swiss albino and Charles River mice (20 to 32 grams) of 705 mg/kg of body weight following intraperitoneal administration in dimethyl sulfoxide. The LD_{50} data for pyrazinamide reported in the Registry of Toxic Effects of Chemical Substances are as follows: mouse, intraperitoneal, 1,680 mg/kg; mouse, subcutaneous, 2,793 mg/kg (RTECS, 1983). No apparent hematological, reproductive, or developmental toxicity occurred in female Swiss CD-1® mice administered 400, 800, or 1,200 mg pyrazinamide/kg body weight by oral gavage for 30 days (NIEHS, 1997). In a subchronic study (Rao *et al*., 1998; NIEHS, 2000), male and female $B6C3F_1$ mice treated with 1,000 or 1,500 mg pyrazinamide/kg body weight by gavage developed increases in liver weights and increases in the incidence and severity of hepatocellular glycogen depletion. Significant alterations did not occur in hematology parameters or body weights.

Roman and Georgian (1977) studied the comparative cytogenetic effects of para-aminosalicylic acid sodium salt, pyrazinamide, and rifampicin in human peripheral blood cultures. The structural chromosomal lesions were randomly distributed between the different chromosome groups of the human karyotype; nearly 20% to 30% of affected cells had more than one lesion per metaphase. In the treatments with sodium para-aminosalicylate and pyrazinamide, the analysis of the frequencies of the cells carrying chromosomal aberrations and of the chromosomal lesion types indicated a dose-response correlation. The *in vitro* studies indicate a potential genetic hazard in the use of these drugs.

STUDY RATIONALE

This study was conducted by the NIEHS as part of its program to evaluate the safety, in pregnant women and in the developing conceptus, of drugs used in the treatment of AIDS or the opportunistic infections accompanying AIDS. The effects on the motility and density of sperm were also evaluated. Tuberculosis is a common opportunistic infection of AIDS patients and is treated with numerous antibacterial compounds, including pyrazinamide, isoniazid, rifampicin, rifabutin, streptomycin, and ethambutol (CDC, 1998a). Considering the numerous antiretroviral agents currently recommended (CDC, 1998b), the potential for drug interactions is enormous. AZT and pyrazinamide are only two of numerous therapeutic compounds an AIDS patient with tuberculosis might receive. Animal reproduction studies have been conducted on AZT alone and on pyrazinamide alone, but not on the combination therapy of AZT plus pyrazinamide. Also, it is not known whether the combination therapy of AZT plus pyrazinamide can cause harm to the conceptus when administered to pregnant women or whether the treatment can affect reproductive capacity. To facilitate the screening of chemicals for reproductive and developmental toxicity, the National Toxicology Program has developed a protocol that combines aspects of other studies designed for this purpose (Morrissey *et al*., 1989; Harris *et al*., 1992). Because the liver has been shown to be a target organ of pyrazinamide in humans, liver enzyme determinations and evaluation of liver weight and pathology were included.

The objective of this study was to obtain controlled laboratory data on the reproductive and developmental toxicity of AZT and pyrazinamide combination therapy. Adult mice were evaluated for clinical signs, body weights, sperm function evaluations, clinical pathology evaluations (selected parameters due to limited amount of sample), and necropsy and histopathology evaluations for selected groups and tissues. Offspring were evaluated for viability, external abnormalities, and weight.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF CHEMICALS

3´-Azido-3´-deoxythymidine (AZT; Lot 7434-36-05 RTI) was manufactured by Raylo Chemicals (Edmonton, Alberta) and Burroughs Wellcome (Research Triangle Park, NC) and supplied as a powder. Pyrazinamide (Lot 7494 40-03 RTI) was manufactured by Aldrich Chemical Co. (Milwaukee, WI) and supplied as a powder. Identification of both compounds was confirmed by infrared and nuclear magnetic resonance spectroscopy. The purity of both AZT and pyrazinamide was determined by high-performance liquid chromatography to be greater than 99%.

The control article was an aqueous solution containing 0.5% methylcellulose. The methylcellulose (Lot 876672) was procured from Fisher Scientific Co. (Pittsburgh, PA).

DOSE FORMULATIONS

The test articles were combined, singly or in combination, with the control article. The dose formulations were then stirred until visually homogeneous. Stability studies at refrigeration and room temperatures indicated a stability for AZT/pyrazinamide formulations of at least 23 days when stored refrigerated or at ambient temperature. Dose formulations for this study were stored refrigerated and protected from light and were used within 20 days of mixing.

Samples at each dose concentration from the initial mix were analyzed for concentration prior to dosing. Residual formulations taken from the same mix after dosing were also analyzed. Prior to dosing, the found concentrations of AZT ranged from 94.5% to 103% of the target concentrations. The found concentrations of pyrazinamide were 90.3% to 111% of the target concentrations. Found concentrations of the residual formulations ranged from 96.4% to 99.9% (AZT) and 95.3% to 111% (pyrazinamide) of the target concentrations.

At the first mix, samples taken from the top, middle, and bottom of selected formulations were analyzed in triplicate to determine homogeneity. Results from these analyses indicated that the formulations were homogeneous.

STUDY DESIGN

Male and female Swiss CD-1® mice were obtained from Charles River Laboratories, Inc., Raleigh, NC. The mice were separated by sex and group housed (five per cage) during quarantine before randomization; all mice were individually housed after randomization, except during cohabitation, when the animals were housed one male and two or three females per cage.

The mice were housed in polycarbonate cages with solid bottoms and sides. The cages were suspended on stainless steel racks and heat-treated hardwood chips were used as the contact bedding. Appropriate environmental conditions were maintained in the animal rooms throughout quarantine and testing.

At terminal sacrifice, blood samples were collected from five sentinel animals per sex from each animal room as part of the animal disease screening program. Results indicated that all animals were free of viral antibodies.

The basic premise for dose selection is that the high-dose level should induce some measurable evidence of toxicity (e.g., anemia, weight loss, target organ toxicity). In a number of studies conducted with this protocol, AZT at the doses selected (200 and 400 mg/kg per day) caused decreases in hematology parameters, increased resorptions, and decreased litter sizes (NIEHS, 1999). The human dose is 10 mg/kg per day (PDR, 1999). The selected doses were 20 times and 40 times human doses, but on a body surface area basis, the doses were close to 2 times and 4 times therapeutic dose (Freireich, et al., 1966).

The pyrazinamide doses selected were 300, 1,500, and 3,000 mg/kg per day. Grosset, et al. (1992) reported eradication of experimental tuberculosis in Swiss CD-1® mice with 150 mg of pyrazinamide/kg per day. In a previous reproductive/developmental and general toxicity study of pyrazinamide in Swiss CD-1® mice (NIEHS, 1997), dosages of pyrazinamide as high as 1,200 mg/kg per day did not result in significant toxicity. A subchronic toxicity study was conducted with pyrazinamide alone and in combination with AZT (Rao et al., 1998; NIEHS, 2000). For the B6C3F1 mice treated with 1,500 mg of pyrazinamide/kg per day alone, significant alterations in in life body weights, survival data, clinical signs, and hematology data were not evident at day 30 or at 13 weeks. Mild liver toxicity in the form of elevated liver weights and hepatocyte hypertrophy were evident at 13 weeks. A mild anemia was evident at both time points in mice treated with 1,500 mg of pyrazinamide/kg + 400 mg of AZT/kg. On a mg/kg per day basis, doses of pyrazinamide selected for the current study were 2, 10, and 20 times the therapeutic dose for experimental tuberculosis in mice (Grosset, et al., 1992). Evidence of mild hepatotoxicity was present at the end of the subchronic study; thus, liver weights and liver enzymes were evaluated.

A summary of the study design is presented in Table 2. The design of this study is a modification of a design published elsewhere (Harris et al., 1992). The oral route of administration was selected because it is the route used in humans, and the study was conducted in Swiss CD-1® mice because this strain is routinely used for

reproductive/developmental toxicity evaluations. Doses of AZT alone, pyrazinamide alone, combinations of AZT and pyrazinamide, or the control article were administered by gavage as a single formulation in an aqueous solution containing methylcellulose. AZT was given at a concentration of 200 or 400 mg/kg per day. Pyrazinamide was given at a concentration of 300, 1,500, or 3,000 mg/kg per day. Total daily doses of 20 mL/kg were divided into two equal doses of 10 mL/kg given approximately 6 hours apart. Mice were divided into three groups as follows:

Male Mice

Ten males were assigned to each dose group. Prior to dosing, male mice were cohabited with female-B mice on study days 0 through 4. Male mice were dosed beginning on study day 5 through the day prior to sacrifice. Males were cohabited with female-A mice on study days 9 to 13 to identify any effects of treatment on mating behavior. On study day 25 or 26, all male mice were weighed, and blood samples were obtained from the retroorbital sinus for hematology and clinical chemistry evaluations. The males were then euthanized with carbon dioxide, a necropsy was conducted, and the left testis and epididymis were collected and prepared for evaluation of sperm parameters as described in the Sperm Function Evaluation section.

Female-A Mice

Twenty females were assigned to each dose group. Female-A mice were dosed beginning on study day 0 through the day prior to sacrifice. Male mice were cohabited with female-A mice on study days 9 to 13 to identify any effects of treatment on mating behavior, fertilization, implantation, or the initial stages of development. During cohabitation periods, the females were examined daily for the presence of a vaginal plug as an indicator of pregnancy. If a vaginal plug was evident, that female was housed individually and that day was designated as day 0 of gestation. At the end of the cohabitation period, all animals were housed individually. Prior to parturition on day 18 of presumed gestation (study days 28 to 32), all female-A mice were weighed, and blood samples were taken from the retroorbital sinus for hematology and clinical chemistry evaluations. The female-A mice were then euthanized with carbon dioxide, and necropsy and caesarean section evaluations were conducted. Live fetuses were removed, weighed, anesthetized on ice, and preserved in Bouin's fixative; dead fetuses were also removed and preserved in Bouin's fixative. The uteri of all sperm-negative females were examined for evidence of unsuccessful pregnancy and then press-plated between two heavy plates of glass to visualize implantation sites. Additional endpoints for all female-A mice included gravid uterine weight, number of implantation sites, resorptions, corpora lutea, and dead and live fetuses. For those dams that delivered pups prior to scheduled caesarean section, the live pups were weighed, sexed, and sacrificed; the uterus for each dam was press-plated, and the same parameters evaluated as for those dams sacrificed as scheduled.

Female-B Mice

Twenty females were assigned to each dose group. Prior to dosing, the group B females were cohabited with males on study days 0 through 4. During the cohabitation period, the females were examined daily for the presence of a vaginal plug as an indicator of pregnancy. If a vaginal plug was evident, that female was housed individually and that day was designated as day 0 of gestation. At the end of the cohabitation period, sperm-negative female-B mice were euthanized with carbon dioxide and discarded without necropsy; all other animals were housed individually. Sperm-positive

female-B mice were assigned evenly across dose groups prior to gestation day 6. Female-B mice were subsequently dosed during gestation days 6 through 15, during the fetal organogenesis period, to identify effects on fetal development. Residual effects on parturition and the beginning of lactation were also evaluated. Beginning on gestation day 16, the bedding material and feeders were no longer changed. From gestation day 17 until the litters were delivered, female-B mice were observed twice daily for evidence of labor or delivery. The day delivery was completed was determined to the nearest day and was designated as postnatal day 0. On postnatal days 0 and 1, dam body weights were recorded along with the number of live and dead pups, the number of male and female pups, the incidence of any external malformations, and live pup weights. Dead pups were discarded. On postnatal day 4, female-B mice, including any that did not deliver, were weighed and blood samples were collected from the retroorbital sinus for hematology determinations. These mice were then euthanized with carbon dioxide, and a complete necropsy was performed. The uterus was removed and press-plated. All pups were weighed, given a thorough external examination for lesions or malformations, and the sex was recorded. The pups were then euthanized with carbon dioxide and saved in Bouin's fixative.

Clinical Pathology

Blood was drawn at terminal sacrifice from all mice for hematology determinations and from male and female-A mice for clinical chemistry determinations. All blood samples were taken from the retroorbital sinus under CO2:O2 (70:30) anesthesia and were collected into tubes containing EDTA (hematology) or no anticoagulant (clinical chemistry). Animals were selected in random order for blood collection and samples were analyzed in the order collected. Erythrocyte, platelet, and leukocyte counts; hematocrit; hemoglobin; mean cell hemoglobin; mean cell volume; mean cell hemoglobin concentration; leukocyte differentials; and erythrocyte and platelet morphologies were determined on whole blood using a Technicon H·1™ automated hematology analyzer. Reticulocyte counts were conducted using a Coulter Model Elite™ Flow Cytometer. Blood smears were prepared to manually verify leukocyte differentials and morphologies if necessary, and platelet and erythrocyte morphological alterations were reported only in the raw data. Alanine aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, aspartate aminotransferase, and total bile acids were determined using the Roche Cobas Fara™ automated analyzer.

Sperm Function Evaluations

The left testis from each male was removed at necropsy and weighed. The left epididymis was weighed, then sperm samples were collected and mixed with modified Tyrode's Solution on two prewarmed slides per animal. Slides were maintained at approximately 37° C and were viewed under a light microscope to assess sperm motility. The distal cauda of the epididymis was weighed then placed in a petri dish containing phosphate buffered saline, and the tissue was teased to release the contents. The final caudal epididymal sperm suspension was incubated for at least 15 minutes. An aliquot was then further diluted with saline solution and placed in a bath of hot water for greater than one minute to kill the sperm. Sperm density was determined using a hemocytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-

buffered saline containing 10% dimethyl sulfoxide. Homogenization resistant spermatid nuclei were counted with a hemocytometer.

Histopathology

A histopathologic examination was performed on tissues listed in Table 2. These tissues were cut (5-µm sections) and representative sections were mounted on glass microscope slides and stained with hematoxylin and eosin (testis stained with PAS). All tissues were examined microscopically by a veterinary pathologist and results were described using a standard nomenclature.

Table 2
Experimental Design and Materials and Methods for the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations in Swiss CD-1® Mice

Study Laboratory
Southern Research Institute, Birmingham, AL

Strain and Species
Swiss CD-1® mice

Animal Source
Charles River Laboratories, Inc., Raleigh, NC

Time Held Before Study
10 days

Average Age When Study Began
86 days

Date of Day 0
2/3/95 for group 1 mice and 2/10/95 for group 2 mice

Date of First Dose
Males: day 5 (2/8/95 for group 1 and 2/15/95 for group 2)
Females-A: day 0 (2/3/95 for group 1 and 2/10/95 for group 2)
Females-B: gestation day 6 (2/10-14/95 for group 1 and 2/17-21/95 for group 2)

Date of Last Dose
Males: day prior to terminal sacrifice
Females-A: day prior to terminal sacrifice
Females-B: gestation day 15 (2/19-23/95 for group 1 and 2/26/95-3/2/95 for group 2)

Days of Cohabitation
Males and Females-A: days 9-13
Males and Females-B: days 0-4
When possible, 1 male and 2-3 females within the same dose group were housed together by consecutive animal number.

Necropsy Dates
Males: days 25-26 (2/28/95-3/1/95 for group 1 and 3/7-8/95 for group 2)
Females-A: days 28-32 (gestation day 18; 3/3-7/95 for group 1 and 3/10-14/95 for group 2)
Females-B: Postnatal day 4 or 5 (2/27/95-3/5/95 for group 1 and 3/6-10/95 for group 2); these dates were also days 24-28 of presumed gestation for sperm-positive mice that did not deliver. Sperm-negative mice were necropsied on day 5 (2/8/95 for group 1 and 2/15/95 for group 2) after cohabitation.

Average Age at Terminal Necropsy
Males: 111-112 days
Females-A: 114-118 days
Females-B: 110-116 days

Size of Study Groups
10 males, 20 females-A, 20 females-B per dose group; each dose group divided into group 1 and group 2

Method of Animal Distribution
Animals were assigned to groups using a stratified weight method and then assigned to study groups in random order. To more evenly distribute the number of successful matings, some female-B mice were reassigned at the end of the cohabitation period and prior to dosing.

Method of Animal Identification
Tail tattoo

Table 2
Experimental Design and Materials and Methods for the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations in Swiss CD-1® Mice

Diet
NIH-07 pelleted feed (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*; fresh feed provided weekly or as needed

Water
Tap water (Birmingham, AL), available *ad libitum* via automatic watering system (Edstrom Industries, Inc., Waterford, WI)

Cages
Polycarbonate cages with solid bottoms and sides (Lab Products, Inc., Maywood, NJ); changed once weekly, except during delivery and lactation periods (gestation day 16 to termination) for female-B mice

Bedding
Heat-treated hardwood chips (Sani-Chips®, P.J. Murphy Forest Products Corporation, Montville, NJ); changed once weekly, except during delivery and lactation periods (gestation day 16 to termination) for female-B mice

Cage Filters
Remay® spun-bonded polyester (Andico, Birmingham, AL); changed once every 2 weeks

Racks
Stainless steel (Lab Products, Maywood, NJ); changed once every 2 weeks

Animal Room Environment
Temperature: 70.7° ± 0.9° F
Relative humidity: 43.7% ± 6.3%
Fluorescent light: 12 hours/day
Room air: minimum of 10 changes/hour

Doses
0 mg AZT + 0 mg pyrazinamide per kg body weight per day
0 mg AZT + 300 mg pyrazinamide per kg body weight per day
0 mg AZT + 1,500 mg pyrazinamide per kg body weight per day
0 mg AZT + 3,000 mg pyrazinamide per kg body weight per day
200 mg AZT + 0 mg pyrazinamide per kg body weight per day
200 mg AZT + 300 mg pyrazinamide per kg body weight per day
200 mg AZT + 1,500 mg pyrazinamide per kg body weight per day
200 mg AZT + 3,000 mg pyrazinamide per kg body weight per day
400 mg AZT + 0 mg pyrazinamide per kg body weight per day
400 mg AZT + 300 mg pyrazinamide per kg body weight per day
400 mg AZT + 1,500 mg pyrazinamide per kg body weight per day
400 mg AZT + 3,000 mg pyrazinamide per kg body weight per day

Type and Frequency of Observation
Mortality/moribundity: twice daily
Clinical findings: once daily
Vaginal plugs: days 10-14 for females-A, days 1-5 for females-B
Body weights: days 3, 5, 9, 13, 17, 21, 23, and sacrifice for males; days 0, 4, 12, 16, 20, 23, 26, and sacrifice for females-A; gestation days 0, 8, 12, 15, and postnatal days 0, 1, and 4 for females-B; postnatal days 0, 1, and 4 for F1 pups.

Clinical Pathology
Hematology evaluations on all animals at terminal sacrifice; clinical chemistry evaluations on males and females-A at terminal sacrifice.

Sperm Function Evaluation
Conducted on all males at terminal sacrifice

Necropsy
Complete necropsies were performed on all breeder animals except sperm-negative females-B. The following were collected at necropsy, saved in formalin (testis saved in Bouin's), and examined histopathologically: males - liver, kidney, lung, heart, spleen, thymus, mandibular and mesenteric lymph nodes, right testis, brain, bone marrow, and gross lesions; liver and right epididymis were weighed; females-A - liver and gross lesions; liver was weighed; females-B - no tissues; pups were examined only grossly at necropsy.

STATISTICAL METHODS

Statistical comparisons were made for all groups against each AZT/vehicle control and the group administered the vehicle alone. Reported significance levels were $P \leq 0.05$ and $P \leq 0.01$. Proportion data (e.g., the incidences of pregnancy, resorption, death, and total resorption) for presumed pregnant mice were analyzed using the Cochran Armitage Test for a Linear Trend in Proportions (Snedecor and Cochran, 1967a) and Fisher's exact test (Siegel, 1956). Paternal and maternal body weight evaluations were analyzed using Bartlett's test of Homogeneity of Variances (Sokal and Rohlf, 1969a) and the Analysis of Variance (Snedecor and Cochran, 1967b). If the Analysis of Variance was significant and appropriate (i.e., it passed Bartlett's test, $P > 0.05$), then Scheffe's test (Scheffe, 1953) was used to identify the statistical significance of individual groups. If the Analysis of Variance was not appropriate ($P \leq 0.05$), the Kruskal-Wallis test (Sokal and Rohlf, 1969b) was used; in cases where statistical significance occurred ($P \leq 0.05$), Dunn's (1964) method of multiple comparisons was used to identify the statistical significance of individual groups. These methods were also used to analyze fetal body weight and pup body weight (per litter), as well as all other evaluations involving continuous data. Observations for delivered and dead fetuses of the female-A dams and fetuses from female-A dams caesarean-sectioned on an estimated day 14 of gestation were excluded from fetal body weight summaries and statistical analyses. Data obtained at caesarean-sectioning and natural delivery were evaluated by the Kruskal-Wallis test (Sokal and Rolf, 1969b). In cases where statistical significance occurred ($P \leq 0.05$), Dunn's (1964) method of multiple comparisons was used to identify statistical significance of individual groups.

For terminal body and organ weight data, group means and standard deviations and organ/body weight ratios were calculated. Mean terminal body weights, mean organ weights, and organ/body weight ratios for each treated group were compared to those of the control group by a two-tailed Student's t-test for each sex. The standard deviations used in the t-tests were obtained by pooling the individual values for the control and treated groups. The reported significance level was $P \leq 0.05$.

For clinical pathology data, group means and standard deviations were calculated and data were evaluated for significance using Dunnett's (1955) test. Reported significance levels were $P < 0.05$ and $P < 0.01$.

Sperm evaluation data were analyzed using a two-way analysis of variance. If the interaction was not statistically significant, averages were taken for each compound over the levels of the second compound, and control and treated group means were compared using either Williams' (1971, 1972) or Dunnett's (1955) multiple comparison procedures. The choice between the two tests was based on the evidence of a dose-related trend in the data as assessed by Jonckheere's test (1954). Williams' test was applied if there was an indication of trend ($P < 0.01$), and Dunnett's test was used in the absence of a trend. Prior to analysis, the outlier test of Dixon and Massey (1951) was employed to detect extreme values, and those that were deemed implausible, due to error, or due to sickness from causes other than test-article toxicity were eliminated. The reported significance levels were $P < 0.05$ and $P < 0.01$.

For histopathology data, the presence of dose effects and interaction between AZT and pyrazinamide was assessed using

methods of generalized linear models (Fahrmeir and Tutz, 1994). The dependent variables used were graded histopathological lesions indexed as 0 through 4. These indices were assumed to follow a multinomial distribution. A cumulative logit link function was used to relate dependent variables to treatment effects. AZT and pyrazinamide dose effects and their interactions were investigated by analysis of deviance (Jonckheere, 1954). Statistical significance of main and interaction effects was assessed using P values obtained from likelihood ratio tests (Dunn, 1964; Shirley, 1977).

RESULTS

SURVIVAL AND CLINICAL FINDINGS

Male Mice

Five male mice died during the study (Table 3). Each of the five had been treated with 3,000 mg pyrazinamide/kg body weight alone or in combination with AZT. All were found dead or were sacrificed moribund on day 6, following the initial dose on day 5. Intubation accidents and toxicity due to the highest dose level of pyrazinamide were considered possible causes of mortality; however, neither possibility was confirmed by histopathological findings. The specific cause of death was not determined. The clinical findings (hypoactive, abnormal breathing, cold to the touch, and pallor) in the two male mice sacrificed in a moribund condition were nonspecific and no other significant clinical findings occurred in any of the other male mice during the study.

Female-A Mice

Four female-A mice died during the study (Table 3), and three of the four had been treated with 200 mg AZT/kg body weight + 3,000 mg pyrazinamide/kg body weight. These three mice were found dead on days 6, 7, and 17, and abnormal clinical findings prior to death did not occur. These deaths were considered possibly related to the 3,000 mg/kg dose of pyrazinamide because five male mice treated with the same dose of pyrazinamide also died. Histopathological lesions confirming pyrazinamide toxicity did not occur, however, and the specific cause of death was not determined.

One female-A mouse treated with 400 mg/kg AZT in combination with 300 mg/kg pyrazinamide was sacrificed moribund on day 18. This mouse was dragging its hindlimbs before death. Histopathological lesions confirming a specific cause of death were not observed.

Four female-A mice treated with 400 mg/kg AZT in combination with 3,000 mg/kg pyrazinamide were pale during the latter stages of the study. None of these mice was pregnant and this clinical finding was believed to reflect anemia. One additional mouse in this group had a red substance in the vagina on days 26 through 29. This mouse had all early resorptions. No other significant clinical signs were observed.

Female-B Mice

Three female-B mice died prior to the end of the study (Table 3). One female-B mouse treated with 300 mg pyrazinamide/kg body weight had hypoactivity, tremors, labored breathing, and was cold to the touch prior to moribund sacrifice on day 21. One female-B mouse treated with 1,500 mg/kg pyrazinamide and one treated with 200 mg/kg AZT + 3,000 mg/kg pyrazinamide were found dead on days 19 and 23, respectively. Adverse clinical signs were not observed in these two mice. Histopathology was not performed on these mice, and the specific cause of death was not determined.

One additional female-B mouse treated with 400 mg/kg AZT + 3,000 mg/kg pyrazinamide had a red substance in the vagina on days 12 through 14 and subsequently had one stillborn pup, two pups of unknown sex and viability, and thirteen implantation sites. No other significant clinical signs were observed in any female-B mice.

TABLE 3
Early Deaths in Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Dose[a]	Number of Deaths	Day of Death[b]	Type of Death	Clinical Findings
Male Mice				
0 + 3,000	1	6	Found dead	None
200 + 3,000	2	6	Found dead	None
		6	Moribund sacrifice	Hypoactive, abnormal breathing, pallor
400 + 3,000	2	6	Moribund sacrifice	Hypoactive, cold to touch, abnormal breathing, pallor
		6	Found dead	None
Female-A Mice				
200 + 3,000	3	6	Found dead	None
		17	Found dead	None
		7	Found dead	None
400 + 300	1	18	Moribund sacrifice	Dragging hindlimbs
Female-B Mice				
0 + 300	1	21	Moribund sacrifice	Hypoactivity, tremors, cold to touch, labored breathing
0 + 1,500	1	19	Found dead	None
200 + 3,000	1	23	Found dead	None

[a] AZT + pyrazinamide in mg/kg per day

[b] Day of death calculated from respective day 0 of study.

BODY WEIGHTS AND ORGAN WEIGHTS

Male Mice

AZT Alone

Administration of 200 or 400 mg AZT/kg body weight had no biologically or statistically ($P \leq 0.05$) significant impact on body weights, body weight gain, epididymal weights, or liver weights (Figures 1 and 2; Tables A1 and A2).

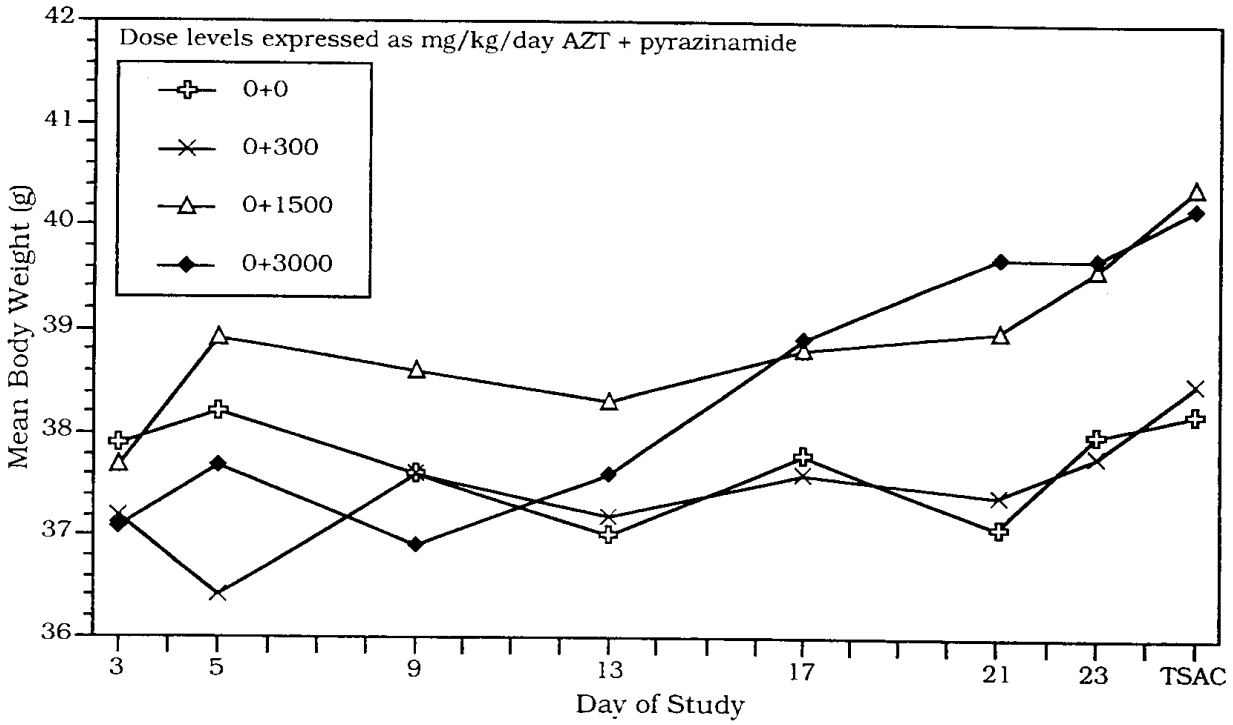
Pyrazinamide Alone

Administration of 300, 1,500, or 3,000 mg pyrazinamide/kg body weight generally resulted in treatment-related elevations in mean body weights and mean liver weights (Figures 1 and 2; Tables A1 and A2). Respective final mean body weights for male mice treated with 1,500 or 3,000 mg/kg were approximately 6% (40.33 grams; $P \leq 0.05$) and 5% (40.1 grams; $P \leq 0.05$) higher than the mean (38.15 grams) in the control group. Mean absolute liver weights for the same treatment groups were approximately 14.2% (2.3780 grams; $P \leq 0.05$) and 13.1% (2.3567 grams; $P \leq 0.05$) higher than the mean (2.0830 grams) in the control group. Although slightly higher than the control group value, the increases in relative liver weights were not statistically significant ($P > 0.05$). A mild decline ($P \leq 0.05$) in the mean relative right epididymal weight occurred in the group treated with 3,000 mg/kg pyrazinamide. The value was approximately 16.7% (1.0 mg/g) lower than the mean (1.2 mg/g) for the controls.

AZT and Pyrazinamide Combinations

Administration of combinations of AZT and pyrazinamide resulted in slight elevations in mean body weights (Figure 1; Table A1). For the male group treated with 400 mg/kg AZT weight + 3,000 mg/kg pyrazinamide, the final mean body weight was approximately 8% (41.06 grams; $P \leq 0.05$) higher than the mean (38.15 grams) in the control group. The minor elevations in body weight in the other combination treatment groups were not statistically significant ($P > 0.05$).

Considerably more dramatic were treatment-related elevations in absolute and relative liver weights (Figure 2; Table A2). Respective mean absolute liver weights for male mice treated with 200 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide were approximately 3.8% (2.1630 grams), 13.2% (2.3570 grams; $P \leq 0.05$), and 21.0% (2.5200 grams; $P \leq 0.05$) higher than the mean (2.0830 grams) in the control group. Mean relative liver weight values (Figure 2) for the same treatment groups were approximately 0.4% (54.8 mg/g), 8.8% (59.4 mg/g; $P \leq 0.05$), and 16.1% (63.4 mg/g; $P \leq 0.05$) higher, respectively, than the mean (54.6 mg/g) for the controls. For the male mice treated with 400 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide, respective mean absolute liver weights were approximately 4.6% (2.1780 grams), 11.9% (2.3300 grams; $P \leq 0.05$), and 22.4% (2.5500 grams; $P \leq 0.05$)



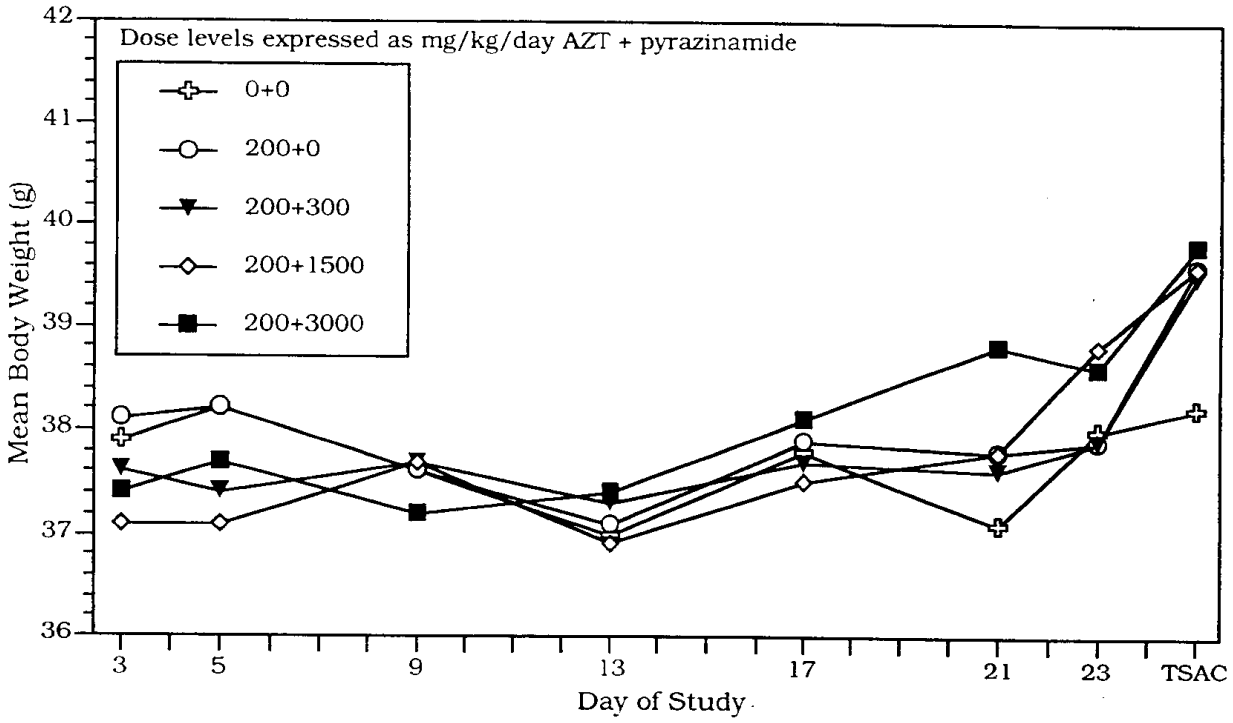


FIGURE 1
Mean Body Weights of Male Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations
(TSAC=Terminal Sacrifice)

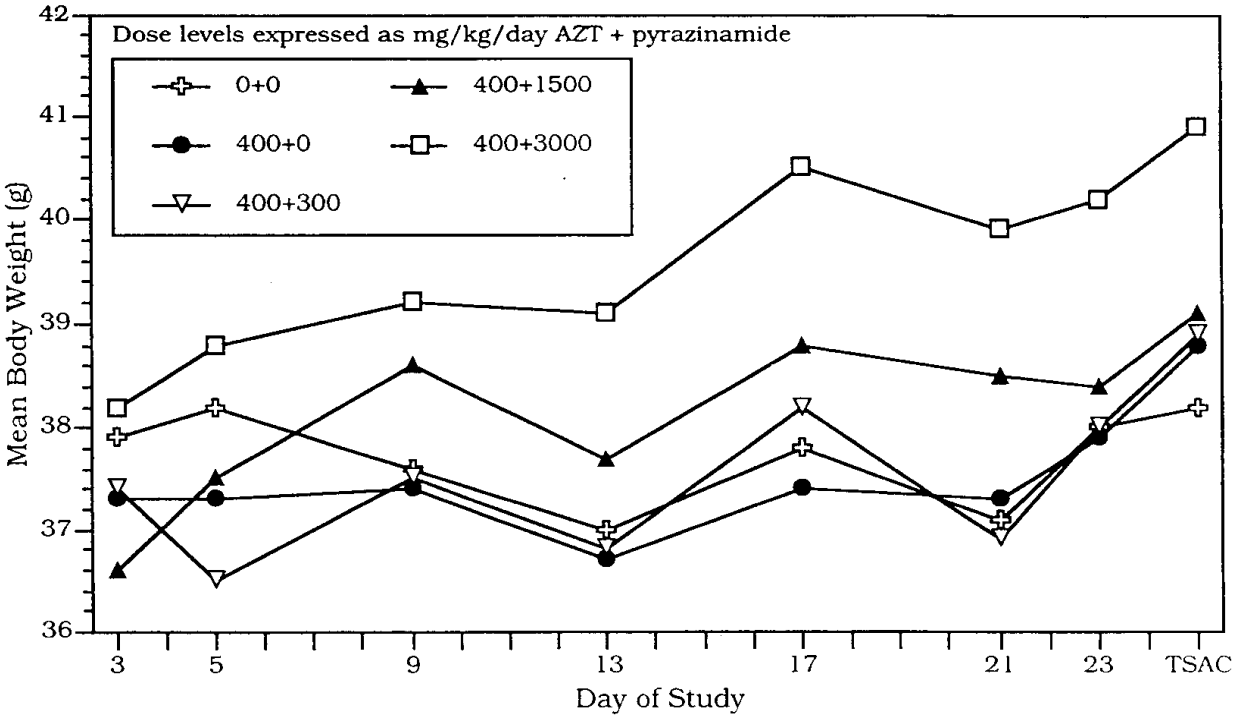


FIGURE 1 (continued)
Mean Body Weights of Male Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations
(TSAC=Terminal Sacrifice)

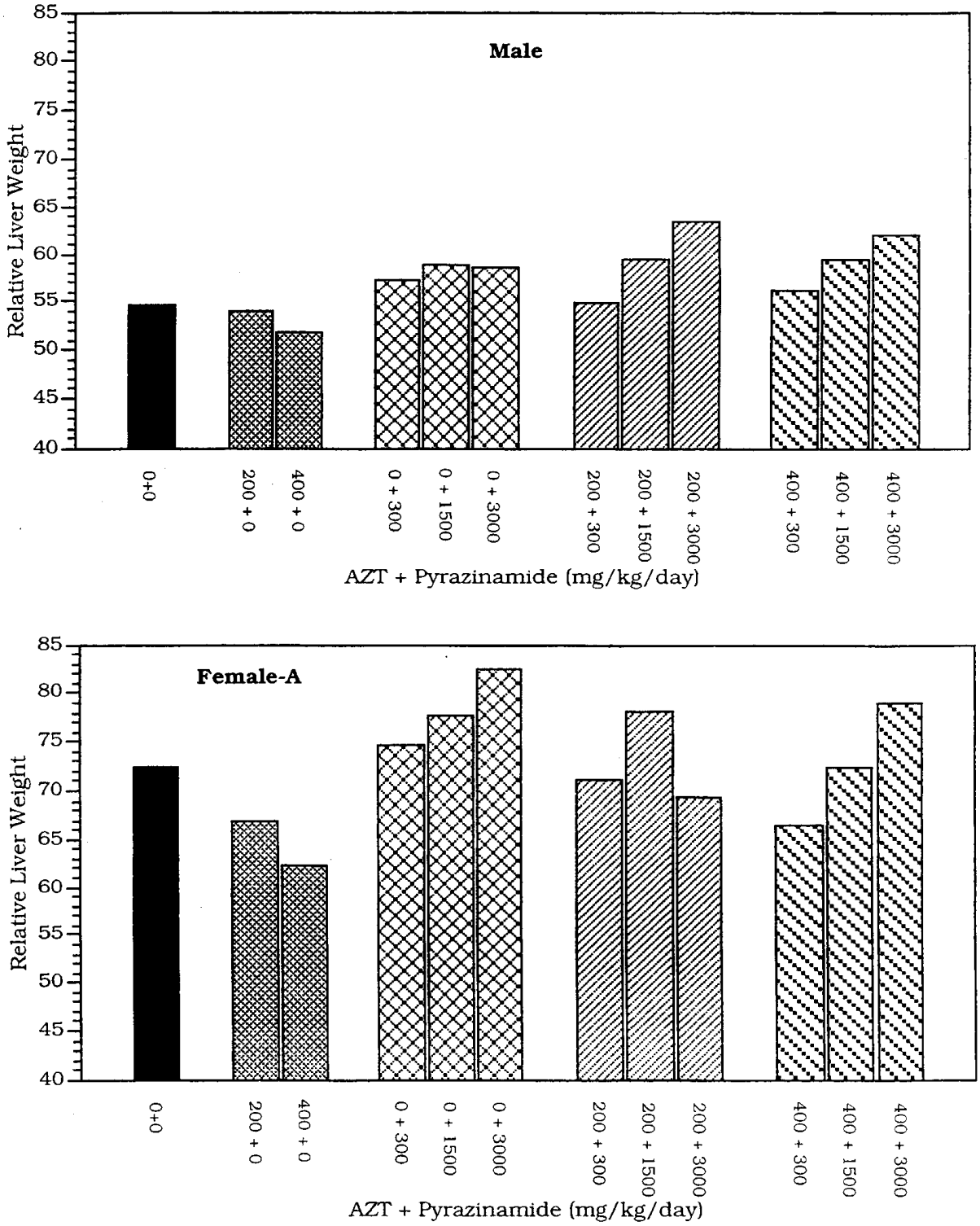


FIGURE 2
Mean Relative Liver Weights for Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

higher than the mean (2.0830 grams) in the control group. Respective mean relative liver weights (Figure 2) for the same treatment groups were approximately 2.7% (56.1 mg/g), 9.0% (59.5 mg/g; P≤0.05), and 13.9% (62.2 mg/g; P≤0.05) higher than the mean (54.6 mg/g) for the controls. Significant (P≤0.05) alterations in right epididymal weights did not occur in any of the male groups treated with combinations of AZT and pyrazinamide.

Female-A Mice

AZT Alone

Female-A mice treated with AZT alone developed dose-related decreases in final mean body weights and gravid uterine weights (Table 4 and Figure 3). Respective mean final body weights in groups treated with 200 or 400 mg AZT/kg body weight were approximately 14% (49.1 grams) and 25% (42.6 grams; P≤0.01) lower than the mean (56.8 grams) in the control group. Gravid uterine weights for the same treatment groups were approximately 35.0% (12.8 grams; P≤0.05) and 66.5% (6.6 grams; P≤0.01) lower than the mean gravid uterine weight (19.7 grams) in the control group. Corrected body weights for female-A mice treated with AZT alone were comparable to those of the control group, indicating that the decreases in final body weights were a reflection of decreased gravid uterine weights subsequent to a reduction in fetal body weights.

Administration of AZT alone to female-A mice resulted in minor dose-related decreases in absolute liver weights (Table A2) and similar dose-related decreases in relative liver weights (Figure 2; Table A2). Respective mean liver weights in female-A groups treated with 200 or 400 mg/kg AZT were approximately 9.9% (2.4387 grams) and 16.6% (2.2581 grams; P≤0.05) lower than the mean (2.7069 grams) in the control group. Respective relative liver weights for the same treatment groups were approximately 7.6% (66.9 mg/g) and 13.9% (62.4 mg/g; P≤0.05) lower than the mean (72.5 mg/g) for the controls.

Pyrazinamide Alone

Administration of 300, 1,500, or 3,000 mg pyrazinamide/kg body weight had no biologically significant impact on body weight gains, final mean body weights, or corrected body weights (Table 4 and Figure 3). Treatment-related declines in gravid uterine weights, however, did occur. Respective mean gravid uterine weights for the 300, 1,500, and 3,000 mg/kg treatment groups were approximately 12.2% (17.3 grams), 21.8% (15.4 grams; not significant, P>0.05, due to high variability), and 14.2% (16.9 grams; P≤0.05) lower than the mean (19.7 grams) for the control group. The declines in gravid uterine weights corresponded with diminished fetal body weights.

TABLE 4
Final Body Weights, Gravid Uterine Weights, and Corrected Body Weights of Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

		Mean Weights (g) ± Standard Deviation		
Dose[a]	N[b]	Final Body	Gravid Uterine	Corrected Body[c]
0 + 0	13	56.8 ± 5.9	19.7 ± 4.9 +	37.2 ± 2.7
200 + 0	15	49.1 ± 8.1	12.8 ± 5.9*	36.3 ± 3.4
400 + 0	16	42.6 ± 7.6**	6.6 ± 5.3**	36.0 ± 3.4
0 + 300	15	54.6 ± 8.4	17.3 ± 7.0	37.3 ± 3.4
0 + 1,500	12	53.4 ± 7.6	15.4 ± 6.1	38.0 ± 2.2
0 + 3,00	13	56.2 ± 3.7	16.9 ± 1.7*	39.3 ± 2.8
200 + 300	14	49.1 ± 8.3	12.0 ± 6.0**	37.0 ± 3.0
200 + 1,500	12	51.0 ± 8.	12.9 ± 5.7*	38.1 ± 2.9
200 + 3,000	5	37.6 ± 4.0*	1.8 ± 2.0*+	35.8 ± 2.0
400 + 300	15	44.1 ± 9.1*	8.2 ± 6.4**	35.9 ± 3.8
400 + 1,500	6	44.7 ± 6.5	7.9 ± 4.7*	36.8 ± 2.6
400 + 3,000	4	39.0 ± 5.9	3.0 ± 3.2	36.0 ± 3.1

[a] AZT + pyrazinamide in mg/kg per day

[b] Number of dams in calculations excludes dams that were not pregnant, that delivered pups prior to scheduled sacrifice, or were sacrificed on an estimated day 14 or day 18 of gestation

[c] Corrected body weight = final body weight – gravid uterine weight

* $P \leq 0.05$ compared to 0 + 0 (control) group by Scheffe's or Dunn's test

** $P \leq 0.01$ compared to 0 + 0 (control) group

+ $P \leq 0.05$ compared to 200 + 0 group

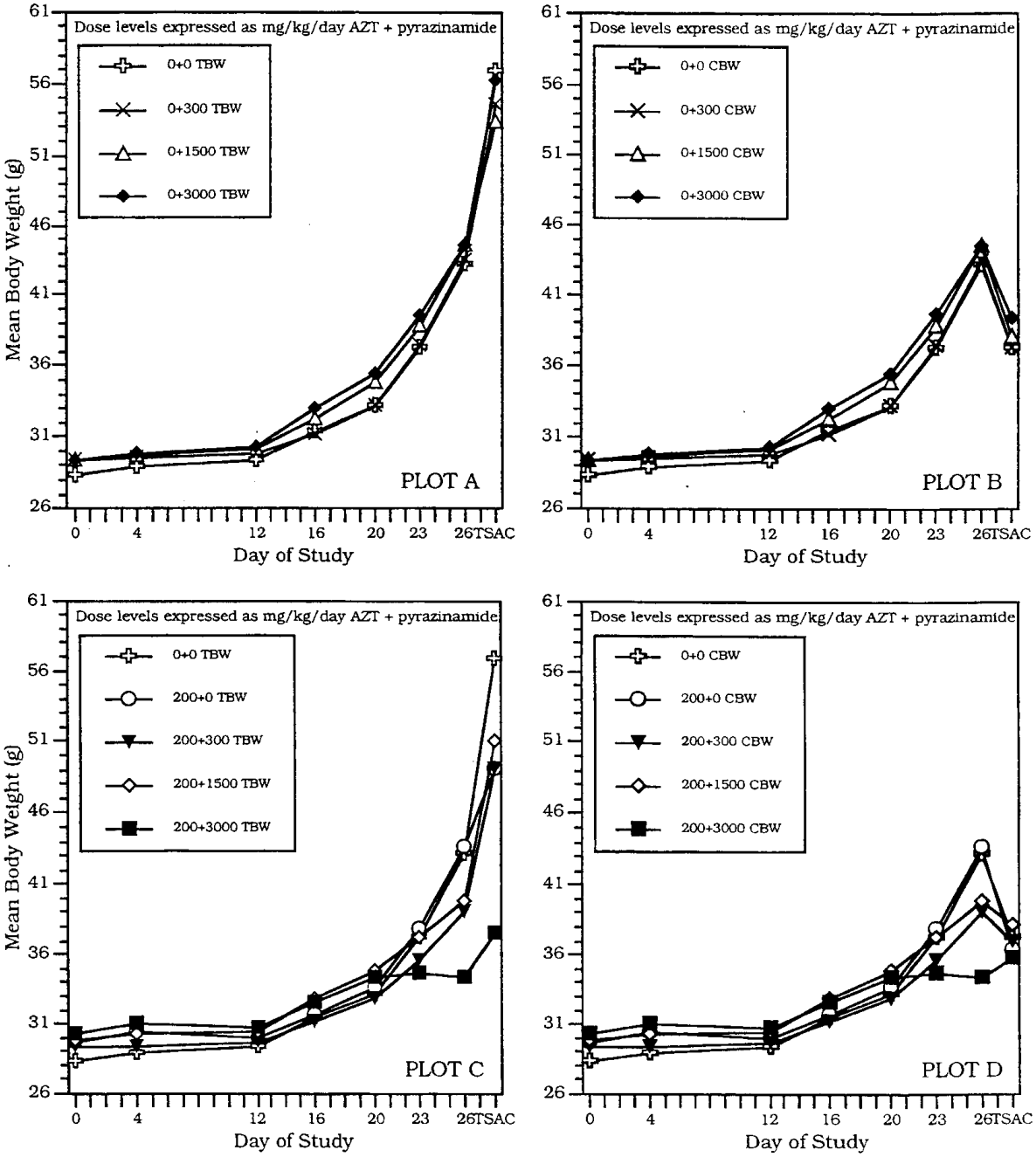


FIGURE 3

Mean Body Weights of Female-A Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

[TBW=Terminal Body Weight prior to caesarean-section; CBW=Corrected Body Weight; (TBW minus gravid uterine weight); TSAC=Terminal Sacrifice; group mean terminal and corrected body weights include only values for dams that were actually pregnant and that were sacrificed and caesarean-sectioned as scheduled on presumed day 18 of gestation.]

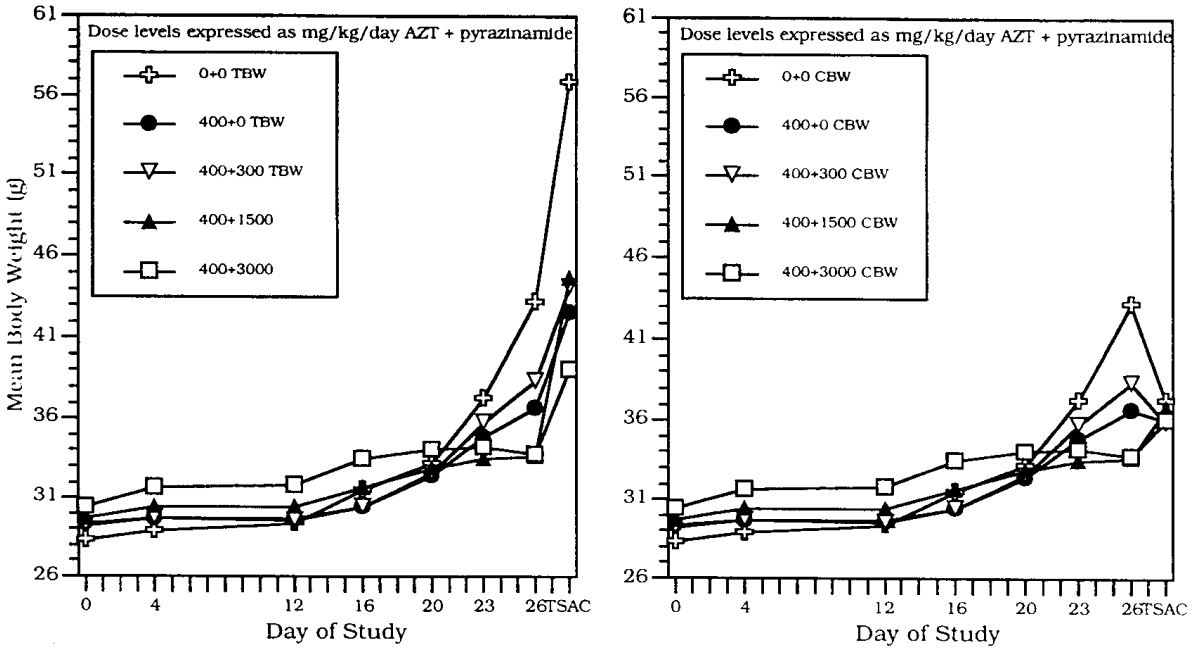


FIGURE 3 (continued)
Mean Body Weights of Female-A Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations
[TBW=Terminal Body Weight prior to caesarean-section; CBW=Corrected Body Weight; (TBW minus gravid uterine weight); TSAC=Terminal Sacrifice; group mean terminal and corrected body weights include only values for dams that were actually pregnant and that were sacrificed and caesarean-sectioned as scheduled on presumed day 18 of gestation.]

Elevated absolute and relative liver weights also occurred in female-A mice treated with pyrazinamide alone (Figure 2; Table A2). Respective absolute liver weights for the groups treated with 300, 1,500, or 3,000 mg/kg pyrazinamide were approximately 2.5% (2.7733 grams), 9.0% (2.9517 grams), and 19.7% (3.2400 grams; $P \leq 0.05$) higher than the mean (2.7069 grams) in the control group. Respective mean relative liver weight values were approximately 2.7% (74.5 mg/g), 7.1% (77.6 mg/g), and 13.8% (82.4 mg/g; $P < 0.05$) higher than the mean (72.5 mg/g) for the controls.

AZT and Pyrazinamide Combinations

Administration of combinations of AZT and pyrazinamide to female-A mice resulted in decreases in mean final body weights. Respective mean final body weights (Table 4 and Figure 3) for female-A mice treated with 200 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide were approximately 14% (49.07 grams), 10% (51.00 grams), and 34% (37.60 grams; $P \leq 0.05$) lower than the mean (56.85 grams) in the control group. For the female-A mice treated with 400 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide, respective mean final body weights were approximately 22% (44.07 grams; $P \leq 0.05$), 21% (44.67 grams), and 31% (39.00 grams) lower than the mean (56.85 grams) for the controls. Lower gravid uterine weights (Table 4) accompanied the declines in final mean body weights. Respective mean gravid uterine weights for female-A mice treated with 200 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide were approximately 39.1% (12.0 grams; $P \leq 0.01$), 34.5% (12.9 grams; $P \leq 0.05$), and 90.9% (1.8 grams; $P \leq 0.05$) lower than the mean (19.7 grams) for the controls. For the female-A groups treated with 400 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide, respective mean gravid uterine weights were approximately 58.4% (8.2 grams; $P < 0.01$), 59.9% (7.9 grams; $P < 0.05$), and 84.8% (3.0 grams; not significant, $P > 0.05$, due to few pregnant mice) lower than the mean (19.7 grams) in the control group. Combination therapy had no impact on corrected final body weights (Table 4) as they were comparable for all treatment groups.

Combination therapy did not result in significant alterations in absolute liver weights (Table A2). For the female-A groups treated with 200 mg/kg AZT + 1,500 mg/kg pyrazinamide and 400 mg/kg AZT + 3,000 mg/kg pyrazinamide, respective mean relative liver weights were 7.7% (78.1 mg/g; $P \leq 0.05$) and 9.1% (79.1 mg/g) higher than the mean (72.5 mg/g) for the controls (Figure 2). Slight declines in mean relative liver weights occurred in all other groups of female-A mice receiving combination therapy.

Female-B Mice

AZT Alone

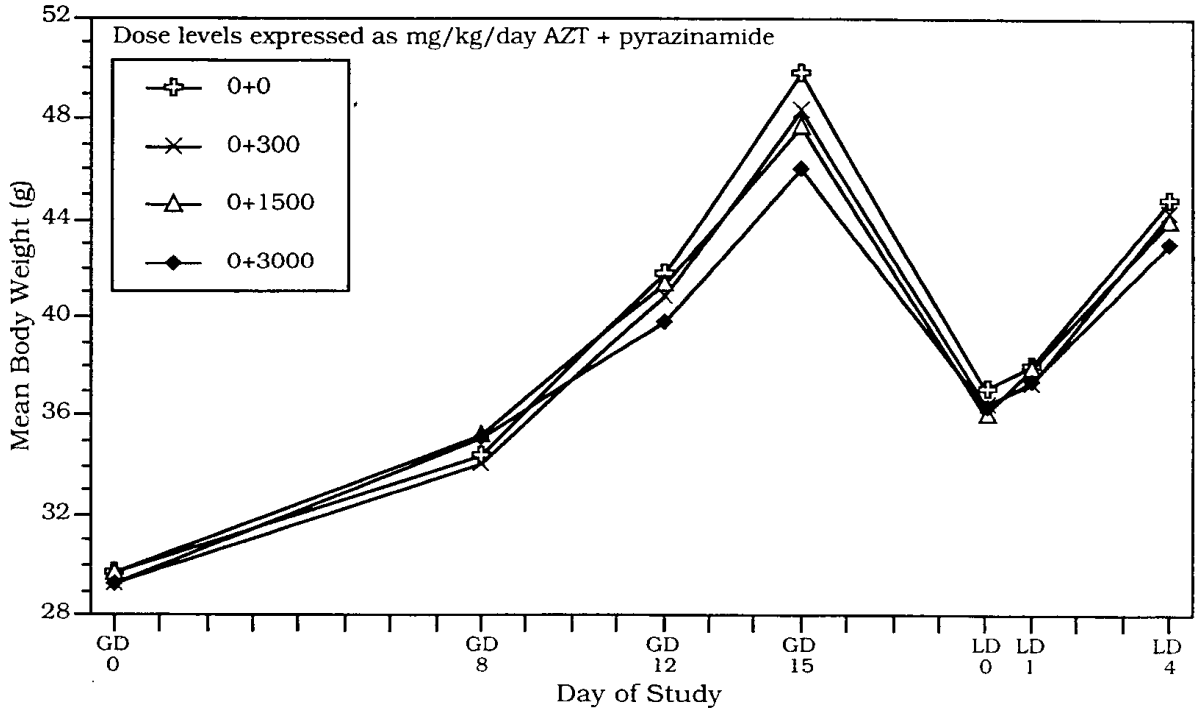
Significant (P≤0.05) alterations in gestational body weights did not occur in female-B mice treated with 200 or 400 mg/kg AZT alone (Figure 4; Table A1). Average body weights and body weight gains during the lactation period did not differ statistically (P>0.05) from those of the control group.

Pyrazinamide Alone

Administration of 300, 1,500, or 3,000 mg/kg pyrazinamide alone to female-B mice did not result in significant alterations (P>0.05) in mean body weights during the gestation or lactation period (Figure 4; Table A1).

AZT and Pyrazinamide Combinations

With the exception of the high-dose combination group, biologically significant alterations in body weights of female-B mice treated with AZT and pyrazinamide did not occur during the gestation or lactation period (Figure 4; Table A1). On day 15 of gestation, the mean body weight for the female-B group treated with 400 mg/kg AZT + 3,000 mg/kg pyrazinamide was approximately 12% (43.7 grams; P≤0.05) lower than the mean (49.8 grams) in the control group. During the lactation period, mean body weights were slightly reduced in female-B mice treated with the higher combinations of AZT and pyrazinamide, but these values were not statistically different (P>0.05) from the control group.



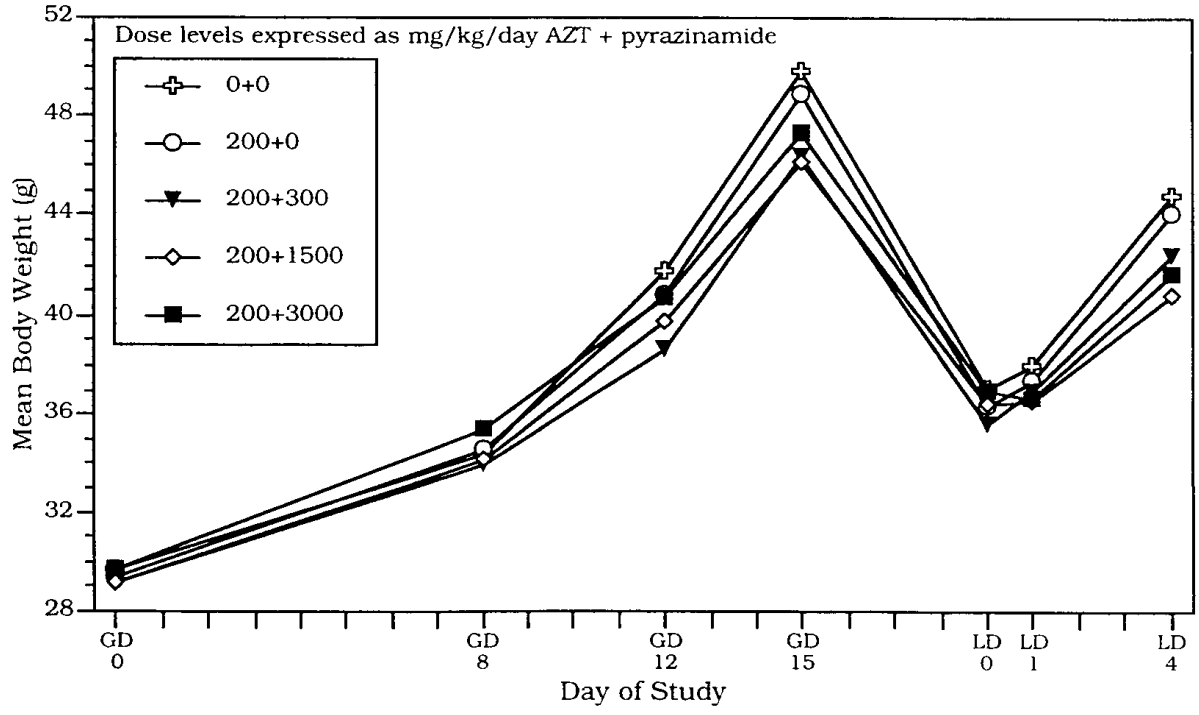


FIGURE 4

Mean Body Weights of Female-B Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

(GD=Gestation Day; LD=Lactation Day; group mean weights include only values for those dams that were actually pregnant, that survived to lactation day 4, and that delivered pups that survived to lactation day 4.)

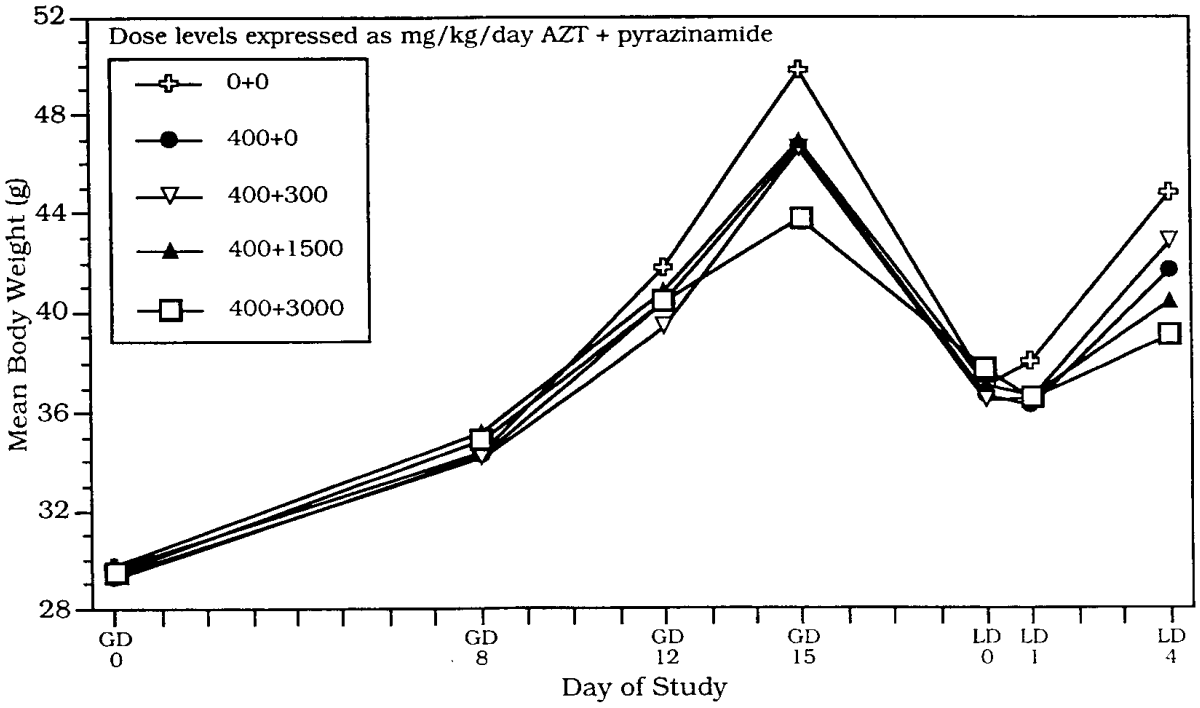


FIGURE 4 (continued)
Mean Body Weights of Female-B Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations
(GD=Gestation Day; LD=Lactation Day; group mean weights include only values for those dams that were actually pregnant, that survived to lactation day 4, and that delivered pups that survived to lactation day 4.)

CLINICAL PATHOLOGY

Hematology

Although it is difficult to compare results between males and females or between groups dosed differently, it appears that the duration of treatment had a major impact on the severity of the hematology results. Severity was greatest in female-A mice dosed for approximately 30 days. Alterations were not as prominent in the males dosed for approximately 20 days, and the female-B groups dosed for approximately 10 days had rather mild alterations. Specific details for male, female-A, and female-B mice are discussed in the following sections.

Male Mice

In general, male mice treated with AZT alone developed a slight macrocytic anemia and thrombocytosis. Administration of pyrazinamide alone had no impact on erythrocyte (RBC) parameters. Combination therapy with AZT and pyrazinamide resulted in a mild to moderate anemia and thrombocytosis, and the severity of the anemia and thrombocytosis was greater than that observed subsequent to treatment with AZT alone.

AZT Alone

Administration of AZT alone to male mice resulted in a slight decline in mean RBC counts (Figure 5; Table B1). Although not statistically significant ($P>0.05$), respective mean RBC counts in male mice treated with 200 or 400 mg AZT/kg body weight were approximately 12% ($8.22 \times 10^6/\mu L$) and 13% ($8.18 \times 10^6/\mu L$) lower than the mean RBC count ($9.37 \times 10^6/\mu L$) in the control group. Reduced hemoglobin (HGB) and hematocrit (HCT) values paralleled the dose-related declines in RBC counts (Table B1). Slight elevations in mean cell volume (MCV) and mean cell hemoglobin (MCH) also accompanied the slight anemia. Respective MCV values for male mice treated with 200 or 400 mg/kg of AZT (Figure 6; Table B1) were approximately 10% (53.2 fL; $P\leq0.01$) and 11% (53.6 fL; $P\leq0.01$) higher than the mean (48.2 fL) in the control group. The MCH values (Table B1) in both treatment groups were approximately 7% (17.5 pg) higher than the mean (16.4 pg) in the control group. Reticulocyte counts were only slightly elevated.

Although not statistically significant ($P>0.05$), platelet counts in both male groups treated with AZT alone were slightly higher than the control group value (Table B1). Respective mean platelet counts in male mice treated with 200 or 400 mg/kg AZT were approximately 1.2 times ($1,147 \times 10^3/\mu L$) and 1.2 times ($1,129 \times 10^3/\mu L$) the mean ($932 \times 10^3/\mu L$) in the control group (Figure 7).

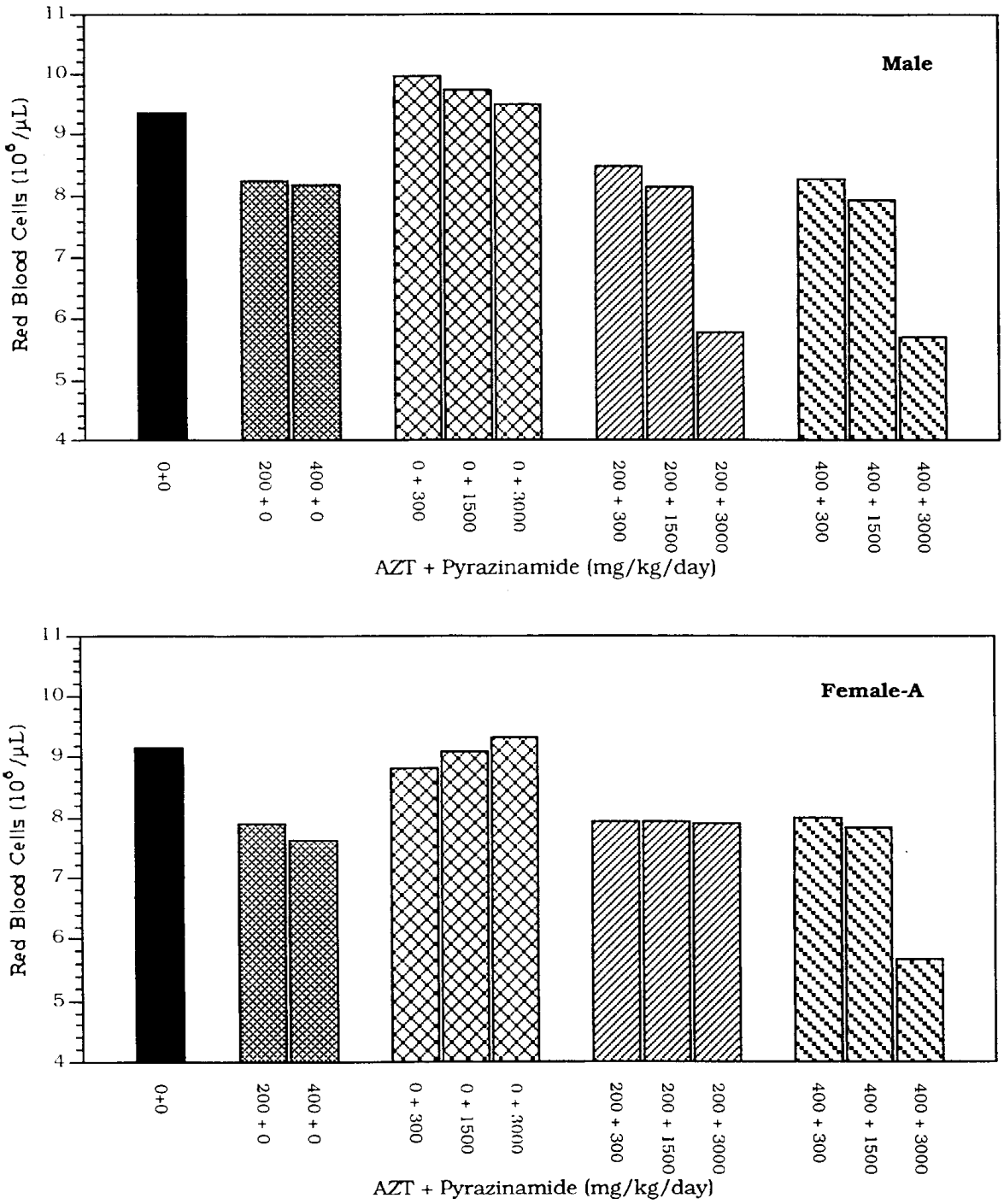


Figure 5
Mean Red Blood Cell (Erythrocyte) Counts for Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

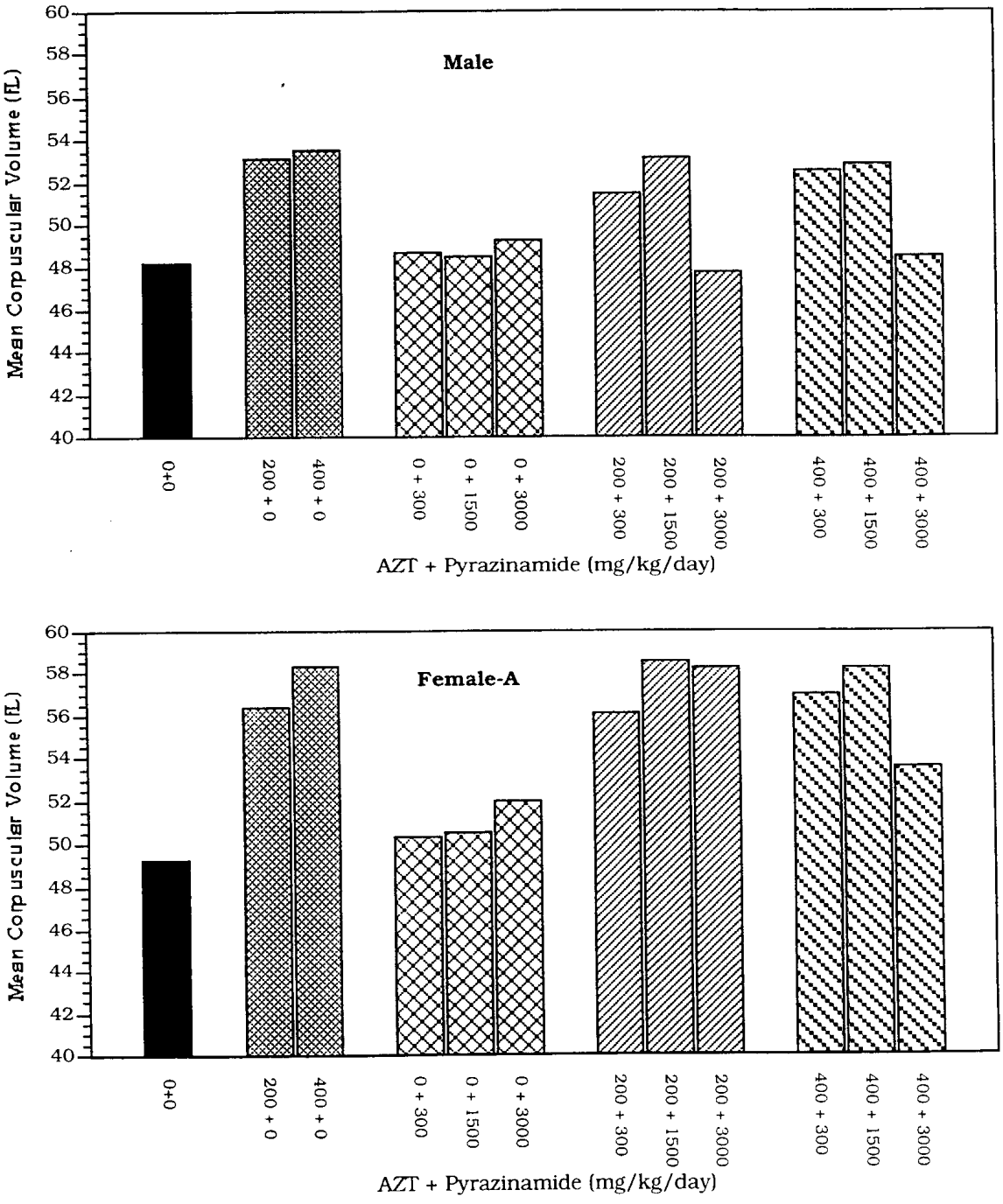


FIGURE 6
Mean Corpuscular Volumes for Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

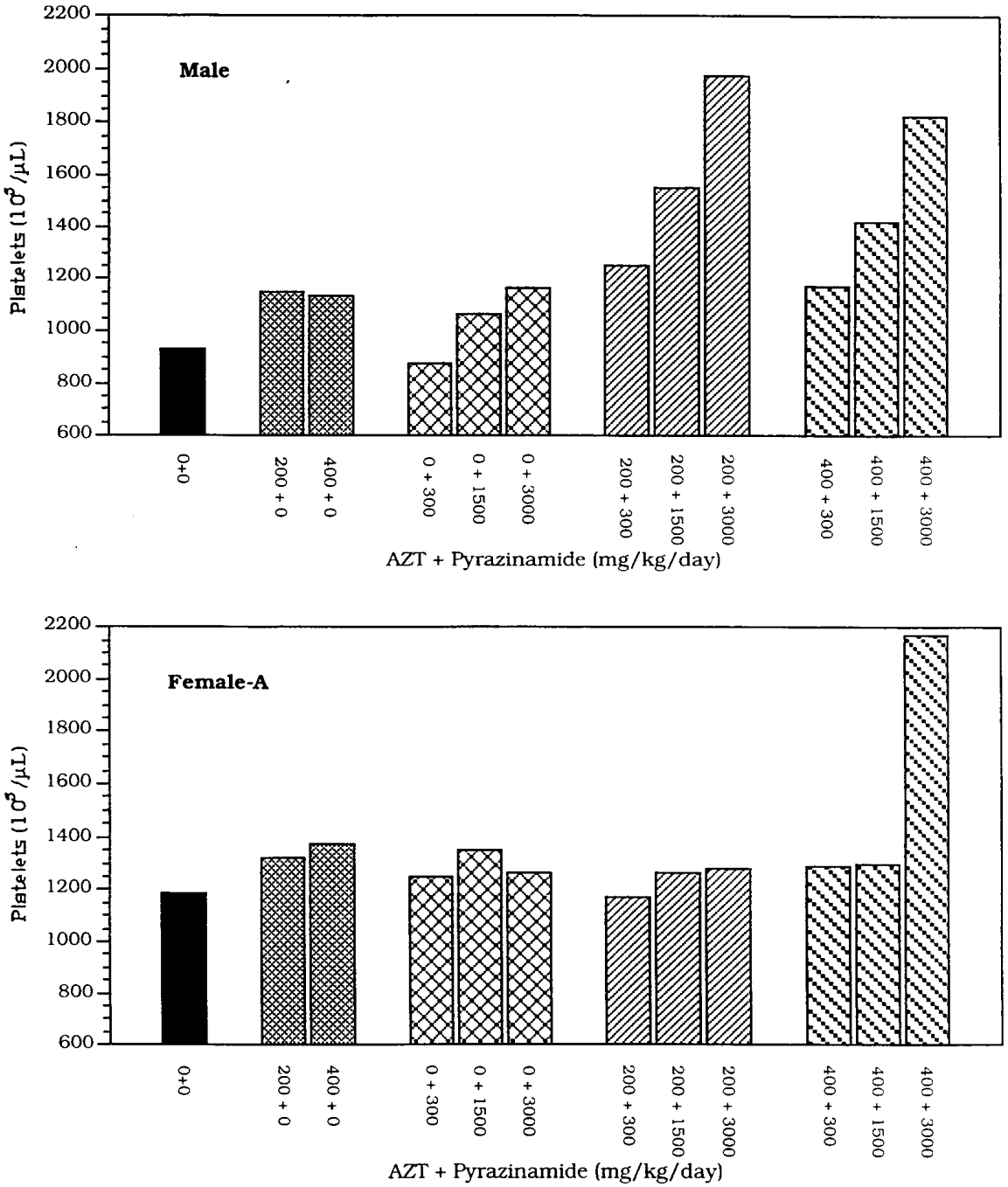


FIGURE 7
Mean Platelet Counts for Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Pyrazinamide Alone

Administration of 300, 1,500, or 3,000 mg/kg pyrazinamide alone to male mice did not result in statistically significant alterations (P>0.05) in any of the hematology parameters (Table B1). Although not statistically significant (P>0.05), mean platelet counts in the two highest dose groups were slightly higher than the platelet count in the controls. Respective mean platelet counts (Figure 7; Table B1) in male mice treated with 1,500 or 3,000 mg/kg pyrazinamide were approximately 1.1 times (1,059 x 10^3/µL) and 1.2 times (1,163 x 10^3/µL) the mean platelet count (932 x 10^3/µL) in the control group.

AZT and Pyrazinamide Combinations

The most significant hematology alterations in male mice treated with combinations of AZT and pyrazinamide consisted of mild to moderate treatment-related anemia and thrombocytosis (Table B1). The severity of the anemia and thrombocytosis was greater than that detected subsequent to treatment with AZT alone. Respective mean RBC counts in male mice (Figure 5) treated with 200 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide were approximately 10% (8.47 x 10^6/µL), 13% (8.14 x 10^6/µL), and 38% (5.78 x 10^6/µL; P≤0.01) lower than the RBC count (9.37 x 10^6/µL) in the control group. Respective mean RBC counts detected in male mice treated with 400 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide (Figure 5) were approximately 12% (8.27 x 10^6/µL), 15% (7.94 x 10^6/µL; P≤0.05), and 39% (5.71 x 10^6/µL; P≤0.01) lower than the mean (9.37 x 10^6/µL) in the controls. Diminished HGB and HCT values paralleled the declines in RBC counts (Table B1). Treatment-related elevations in MCV and MCH values occurred only in male groups treated with AZT + 300 or 1,500 mg/kg pyrazinamide (Table B1). Respective MCV values in male mice (Figure 6) treated with 200 mg/kg AZT + 300 or 1,500 mg/kg of pyrazinamide were approximately 7% (51.5 fL) and 10% (53.2 fL; P≤0.01) higher than the MCV value (48.2 fL) in the control group. Respective MCV values in male mice (Figure 6) treated with 400 mg/kg AZT + 300 or 1,500 mg/kg pyrazinamide were approximately 9% (52.6 fL; P≤0.05) and 10% (52.9 fL; P≤0.01) higher than the mean (48.2 fL) in the controls. Although not statistically significant (P>0.05), respective MCH values (Table B1) in male mice treated with 200 mg/kg AZT + 300 or 1,500 mg/kg pyrazinamide were approximately 5% (17.3 pg) and 7% (17.6 pg) higher than the mean (16.4 pg) in the controls. MCH values for both groups treated with 400 mg/kg AZT + 300 or 1,500 mg/kg pyrazinamide were approximately 7% (17.5 pg) higher than the mean (16.4 pg) in the controls. Significant (P≤0.05) elevations in MCV and MCH values did not occur in male mice treated with 200 or 400 mg/kg AZT + 3,000 mg/kg pyrazinamide.

Mild declines in reticulocyte counts accompanied the anemia in the male groups treated with AZT in combination with the highest dose of pyrazinamide (Table B1). Although not statistically significant (P>0.05), respective reticulocyte counts (Figure 8) in male mice treated with 200 or 400 mg/kg AZT + 3,000 mg/kg pyrazinamide were

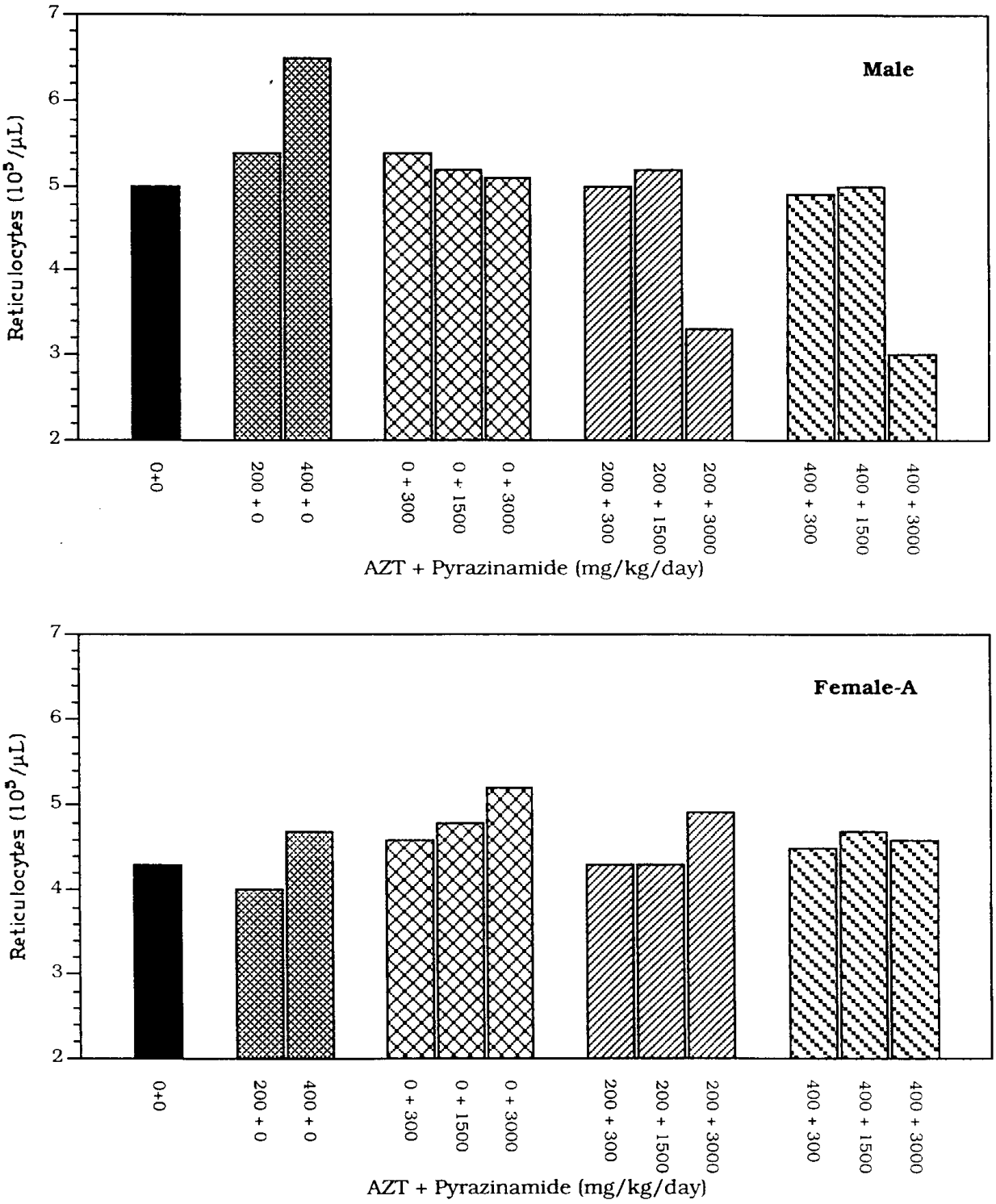


FIGURE 8
Mean Reticulocyte Counts for Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

approximately 34% (3.3 x 10^5/µL) and 40% (3.0 x 10^5/µL) lower than the mean (5.0 x 10^5/µL) in the controls. Biologically significant alterations in reticulocyte counts did not occur in the male groups treated with AZT in combination with 300 or 1,500 mg/kg pyrazinamide (Table B1).

A distinct dose-related elevation in platelet counts (Table B1) occurred in male mice treated with combinations of AZT and pyrazinamide, and the severity of the thrombocytosis was considerably greater than that in male mice treated with either compound alone. Respective mean platelet counts in male groups (Figure 7) treated with 200 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide were approximately 1.3 times (1,247 x 10^3/µL), 1.7 times (1,553 x 10^3/µL; P≤0.01), and 2.1 times (1,978 x 10^3/µL; P≤0.01) the mean (932 x 10^3/µL) in the control group. Respective mean platelet counts in male mice (Figure 7) treated with 400 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide were approximately 1.2 times (1,172 x 10^3/µL), 1.5 times (1,420 x 10^3/µL; P≤0.01), and 2 times (1,824 x 10^3/µL; P≤0.01) the mean platelet count (932 x 10^3/µL) for the controls.

Although not statistically significant (P>0.05), biologically significant declines in total leukocyte (WBC) counts (Table B1) occurred in male mice treated with 200 or 400 mg/kg AZT + 3,000 mg/kg pyrazinamide. Respective mean WBC counts for these treatment groups (Figure 9) were approximately 20% (4.93 x 10^3/µL) and 42% (3.58 x 10^3/µL) lower than the mean WBC count (6.19 x 10^3/µL) in the male control group. Evaluation of the differential data revealed declines in neutrophil and lymphocyte counts (Table B1). Respective mean neutrophil counts in male mice (Figure 10) treated with 200 or 400 mg/kg AZT + 3,000 mg/kg of pyrazinamide were approximately 19% (0.81 x 10^3/µL) and 23% (0.77 x 10^3/µL) lower than the mean neutrophil count (1.00 x 10^3/µL) in the controls. Respective mean lymphocyte counts for the same treatment groups were approximately 22% (3.72 x 10^3/µL) and 49% (2.44 x 10^3/µL) lower than the mean (4.77 x 10^3/µL) in the controls.

Female-A Mice

In general, the hematological alterations induced by AZT alone were slightly more severe in female-A mice than in males. Administration of pyrazinamide alone to female-A mice did not result in biologically significant hematological changes. Combination therapy with AZT and pyrazinamide resulted in a prominent anemia, leukopenia, and thrombocytosis. As with the males, the severity of these hematological alterations was greater than that induced by AZT alone.

AZT Alone

Administration of AZT alone to female-A mice resulted in a mild anemia (Table B1). Respective mean RBC counts detected in female-A mice (Figure 5) treated with 200 or 400 mg AZT/kg body weight were approximately

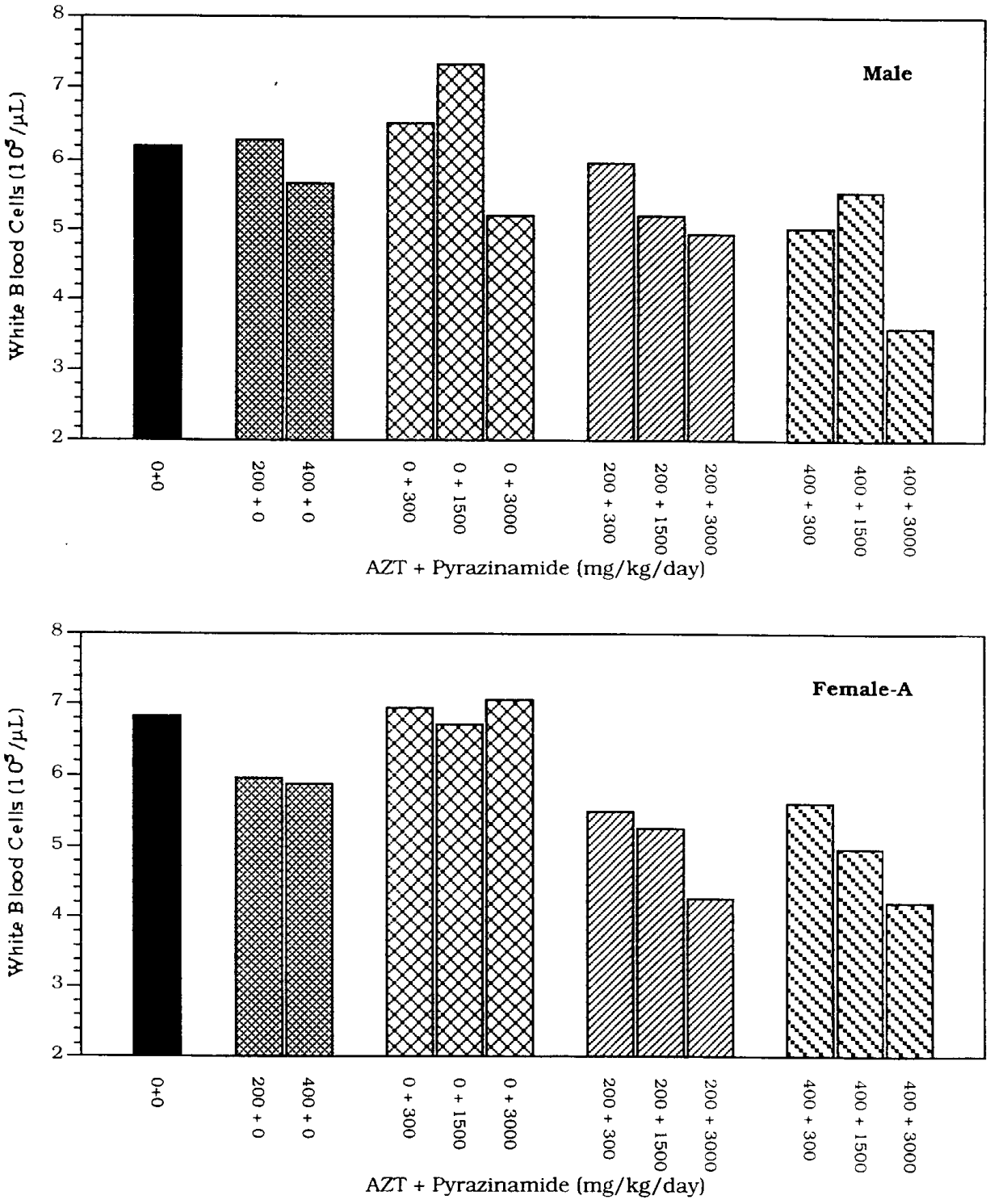


FIGURE 9
Mean White Blood Cell (Leukocyte) Counts for Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

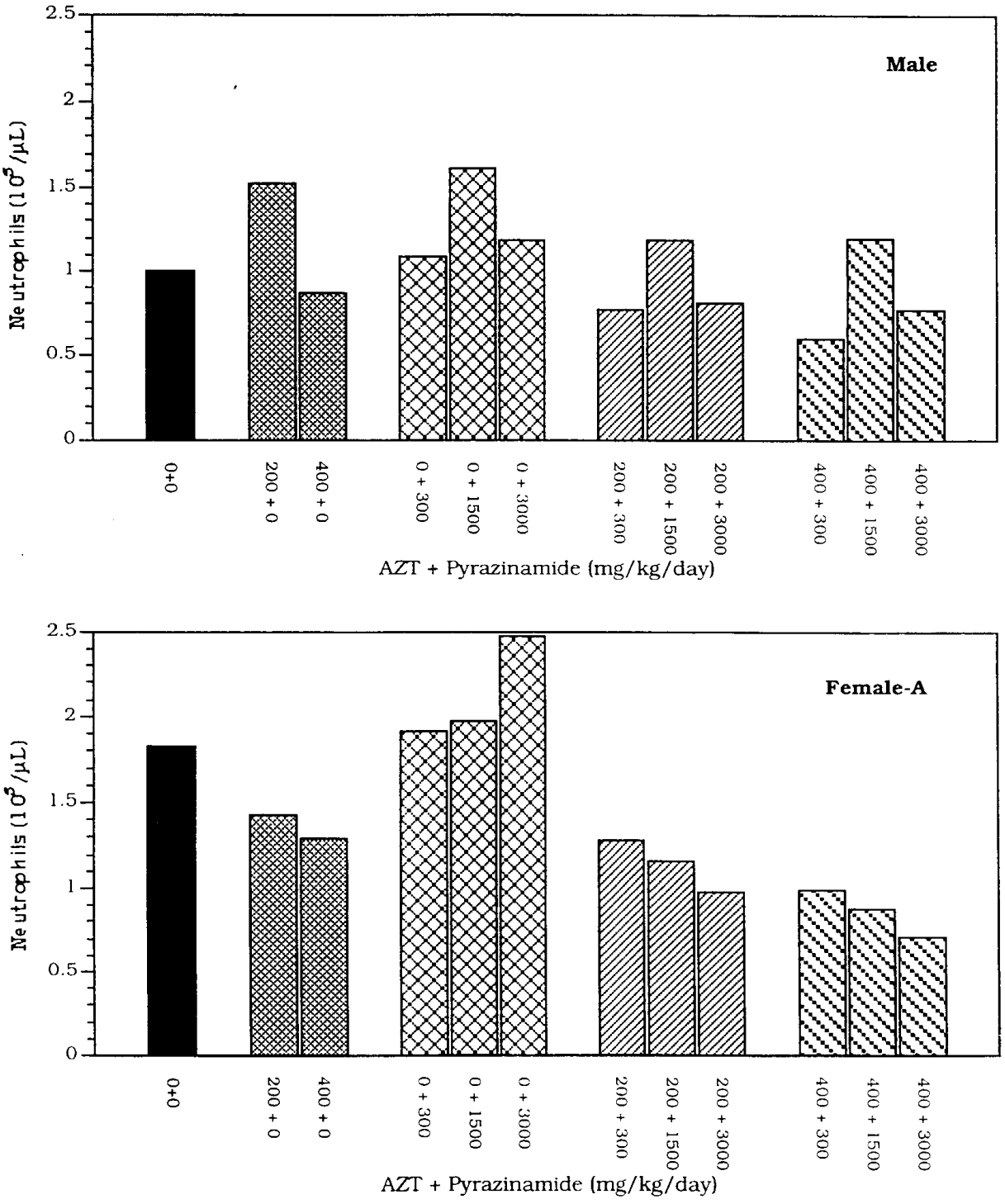


FIGURE 10
Mean Neutrophil Counts for Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

14% (7.88 x 10^6/µL; P≤0.01) and 17% (7.62 x 10^6/µL; P≤0.01) lower than the mean RBC count (9.14 x 10^6/µL) in the controls. Mild declines in HGB and HCT values (Table B1) paralleled the dose-related decline in RBC counts. The anemia was accompanied by dose-related elevations in MCV and MCH values (Table B1). Respective MCV values (Figure 6) in female-A mice treated with 200 or 400 mg AZT/kg body weight were approximately 14% (56.4 fL; P≤0.01) and 18% (58.3 fL; P≤0.01) higher than the MCV value (49.3 fL) in the controls. Respective MCH values (Table B1) for the same treatment groups were approximately 12% (18.4 pg; P≤0.01) and 14% (18.8 pg; P≤0.01) higher than the mean (16.5 pg) in the controls. Reticulocyte counts (Figure 8; Table B1) were within a normal range.

Although not statistically significant (P>0.05), platelet counts were slightly elevated in female-A mice treated with AZT alone (Figure 7; Table B1). Respective mean platelet counts in groups treated with 200 or 400 mg/kg AZT were approximately 1.1 times (1,319 x 10^3/µL) and 1.2 times (1,378 x 10^3/µL) the mean (1,190 x 10^3/µL) in the controls.

Although not statistically significant (P>0.05), a slight leukopenia also occurred in female-A mice treated with AZT alone (Table B1). Respective mean WBC counts (Figure 9) in groups treated with 200 or 400 mg/kg AZT were approximately 13% (5.95 x 10^3/µL) and 14% (5.86 x 10^3/µL) lower than the mean (6.85 x 10^3/µL) in the control group. Evaluation of the differential data revealed a slight neutropenia (Table B1). Respective mean neutrophil counts (Figure 10) in female-A mice treated with 200 or 400 mg/kg AZT were approximately 22% (1.43 x 10^3/µL) and 30% (1.29 x 10^3/µL) lower than the mean neutrophil count (1.83 x 10^3/µL) in the control group.

Pyrazinamide Alone

Biologically significant alterations did not occur in any of the hematology parameters in female-A mice treated with pyrazinamide alone.

AZT and Pyrazinamide Combinations

Combination therapy with AZT and pyrazinamide in female-A mice resulted in treatment-related anemia, thrombocytosis, and leukopenia (Table B1).

The anemia induced by combination therapy in female-A mice was similar in severity to the anemia caused by AZT alone except for the group treated with the highest combination. Respective mean RBC counts in female-A mice (Figure 5; Table B1) treated with 200 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide were approximately 13% (7.94 x 10^6/µL; P≤0.01), 13% (7.91 x 10^6/µL; P≤0.01), and 14% (7.88 x 10^6/µL; P≤0.01) lower than the mean

(9.14 x 10^6/µL) in the control group. Respective mean RBC counts in female-A mice (Figure 5) treated with 400 mg/kg AZT + 300, 1,500, or 3,000 mg/kg of pyrazinamide were approximately 13% (7.98 x 10^6/µL; P≤0.01), 14% (7.82 x 10^6/µL; P≤0.01), and 38% (5.69 x 10^6/µL; P≤0.01) lower than the mean (9.14 x 10^6/µL) in the controls. Diminished HGB and HCT values (Table B1) accompanied the declines in RBC counts. With the exception of the group receiving the highest combination dose, marked elevations in MCV and MCH values (Table B1) accompanied the anemia. The elevated MCV values occurred in the absence of concurrent elevations in reticulocyte counts (Table B1). Respective MCV values (Figure 6) for female-A mice treated with 200 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide were approximately 14% (56.1 fL; P≤0.01), 19% (58.5 fL; P≤0.01), and 18% (58.2 fL; P≤0.01) higher than the mean (49.3 fL) in the controls. For the groups treated with 400 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide, respective MCV values (Figure 6) were approximately 16% (57.0 fL; P≤0.01), 18% (58.2 fL; P≤0.01), and 9% (53.6 fL; P≤0.01) higher than the mean (49.3 fL) in the controls. Respective MCH values (Table B1) in female-A mice treated with 200 mg/kg AZT + 300, 1,500, or 3,000 mg/kg of pyrazinamide were approximately 14% (18.8 pg; P≤0.01), 15% (19.0 pg; P≤0.01), and 16% (19.2 pg; P≤0.01) higher than the mean (16.5 pg) in the control group. For the groups treated with 400 mg/kg of AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide, respective MCH values were approximately 14% (18.8 pg; P≤0.01), 16% (19.1 pg; P≤0.01), and 8% (17.8 pg; P≤0.01) higher than the mean (16.5 pg) for the controls. The reason for the lower MCV and MCH values in the highest dose combination group was not evident. Reticulocyte counts were within a normal range.

A prominent thrombocytosis occurred in the highest dose combination group (Table B1). For the female-A group treated with 400 mg/kg AZT + 3,000 mg/kg pyrazinamide, the mean platelet count (Figure 7) was approximately 1.8 times (2,166 x 10^3/µL; P≤0.01) the mean (1,190 x 10^3/µL) in the control group. Mean platelet counts in all other female-A combination groups were within 10% of the mean in the controls.

A treatment-related leukopenia occurred in female-A mice receiving combination therapy, and the severity of this leukopenia was greater than that in groups treated with AZT alone (Table B1). Respective mean WBC counts in female-A mice (Figure 9) treated with 200 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide were approximately 20% (5.49 x 10^3/µL), 23% (5.25 x 10^3/µL; P≤0.05), and 38% (4.28 x 10^3/µL; P≤0.01) lower than the mean (6.85 x 10^3/µL) in the control group. For the female-A groups treated with 400 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide, respective mean WBC counts (Figure 9) were approximately 18% (5.61 x 10^3/µL), 27% (4.97 x 10^3/µL; P≤0.01), and 39% (4.21 x 10^3/µL; P≤0.01) lower than the mean (6.85 x 10^3/µL) for the controls. Evaluation of the differential data revealed treatment-related declines in neutrophils and lymphocytes (Table B1). Respective mean neutrophil counts (Figure 10) for female-A mice treated with 200 mg/kg of AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide were approximately 30%

(1.28 x 10^3/µL), 37% (1.16 x 10^3/µL), and 46% (0.98 x 10^3/µL; P≤0.05) lower than the mean neutrophil count (1.83 x 10^3/µL) in the control group. For the female-A groups treated with 400 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide, respective mean neutrophil counts were approximately 46% (0.99 x 10^3/µL; P≤0.05), 52% (0.88 x 10^3/µL; P≤0.01), and 61% (0.71 x 10^3/µL; P≤0.01) lower than the mean (1.83 x 10^3/µL) in the controls. Respective mean lymphocyte counts (Table B1) for female-A mice treated with 200 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide were approximately 15% (3.93 x 10^3/µL), 19% (3.74 x 10^3/µL), and 34% (3.02 x 10^3/µL) lower than the mean (4.61 x 10^3/µL) in the control group. For the groups treated with 400 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide, respective mean lymphocyte counts were approximately 6% (4.32 x 10^3/µL), 19% (3.74 x 10^3/µL), and 30% (3.23 x 10^3/µL) lower than the mean (4.61 x 10^3/µL) in the controls. Although only a small part of the differential count, monocytes were lower in all female-A groups treated with combination therapy (Table B1). Monocyte counts ranged from 19% (0.17 x 10^3/µL) to 38% (0.13 x 10^3/µL; P≤0.05) lower than the mean (0.21 x 10^3/µL) in the control group. Biologically significant alterations did not occur in any of the other differential leukocyte parameters.

Female-B Mice

In general, hematological alterations in female-B mice treated for a shorter duration (approximately 10 days) were considerably less than those described for female-A mice. Anemia did not occur in female-B mice treated with AZT alone, pyrazinamide alone, or combinations of AZT and pyrazinamide.

AZT Alone

Biologically and statistically (P≤0.05) significant alterations in female-B mice treated with 200 or 400 mg AZT/kg body weight were limited to a mild elevation in the MCV value in the high dose group (Table B1). The mean MCV value in the female-B group treated with 400 mg/kg AZT was approximately 5% (53.6 fL; P≤0.05) higher than the mean (51.1 fL) in the control group. No other hematological alterations occurred.

Pyrazinamide Alone

Administration of 300, 1,500, or 3,000 mg pyrazinamide/kg body weight to female-B mice did not result in any biologically or statistically (P≤0.05) significant alterations in any of the hematology parameters.

AZT and Pyrazinamide Combinations

Hematological alterations in female-B mice treated with combination therapy were limited to minor elevations in MCV and MCH values. Anemia did not occur. For the female-B mice treated with 200 mg/kg AZT + 1,500 or 3,000 mg/kg pyrazinamide, respective MCV values (Table B1) were approximately 9% (55.5 fL; P≤0.01) and 7%

(54.6 fL; P≤0.01) higher than the mean (51.1 fL) in the control group. Administration of 400 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide resulted in respective MCV values approximately 6% (54.3 fL; P≤0.01), 5% (53.8 fL; P≤0.01), and 4% (53.2 fL) higher than the mean (51.1 fL) in the controls. The reason for the lower value in the highest-dose combination group was not apparent. Elevations in MCH values (Table B1) were less dramatic, with increases in only three groups. Respective MCH values in female-B mice treated with 200 mg/kg AZT + 1,500 or 3,000 mg/kg pyrazinamide were approximately 8% (18.3 pg; P≤0.01) and 6% (18.1 pg; P≤0.01) higher than the mean (17.0 pg) in the controls. The MCH value in the group treated with 400 mg/kg AZT + 1,500 mg/kg pyrazinamide was approximately 5% (17.9 pg; P≤0.01) higher than the mean (17.0 pg) in the control group. Biologically significant alterations did not occur in RBC, WBC, reticulocyte, or platelet values of any of the female-B groups receiving combination therapy.

Clinical Chemistry

Administration of 200 or 400 mg AZT/kg body weight, 300, 1,500, or 3,000 mg pyrazinamide/kg body weight, or combinations of AZT and pyrazinamide to male and female-A mice did not result in biologically or statistically (P≤0.05) significant alterations in clinical chemistry parameters (Table B2).

NECROPSY OBSERVATIONS

The only lesion found at necropsy believed possibly related to treatment was an enlarged spleen observed in a single male mouse treated with 200 mg/kg AZT + 3,000 mg/kg pyrazinamide. Bone deformity (thoracic vertebrae or ribs) occurred randomly in male and female mice from a variety of treatment groups and may have been secondary to the physical effect of twice daily gavage dosing. Other lesions such as small thymus, small testis, focus on the testis, focus on the liver, small epididymis, and hemorrhage in subcutaneous tissue occurred sporadically with a low incidence and were considered to be spontaneous and unrelated to treatment.

HISTOPATHOLOGIC OBSERVATIONS

Representative microscopic lesions resulting from administration of AZT and pyrazinamide are listed in Table 5 and illustrated in Plates 1 to 6. Target tissues included the bone marrow, liver, and spleen of male mice and the liver of female-A mice. Statistical results include dose-response effects of AZT and pyrazinamide compared to the vehicle control group as well as significant dose-response effects in the presence of fixed levels of AZT and pyrazinamide.

TABLE 5
Representative Microscopic Lesions (Photomicrographs) Related to Treatment in Adult Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Dose[a]	Tissue	Magnification	Plate Number	Lesion
0 + 0	Liver	H160	1	None (control)
400 + 3,000	Liver	H160	2	Glycogen depletion, moderate
0 + 0	Spleen	H160	3	None (control)
400 + 3,000	Spleen	H160	4	Hematopoietic cell proliferation, moderate
0 + 0	Bone Marrow	H160	5	None (control)
400 + 3,000	Bone Marrow	H160	6	Cellular depletion, mild

[a] AZT + pyrazinamide in mg/kg per day

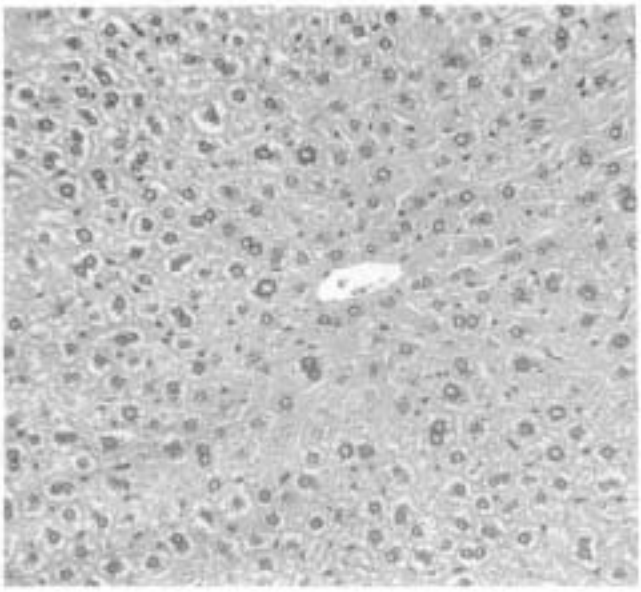


PLATE 1
Liver from a control male mouse showing normally glycogenated hepatocytes. (x160)

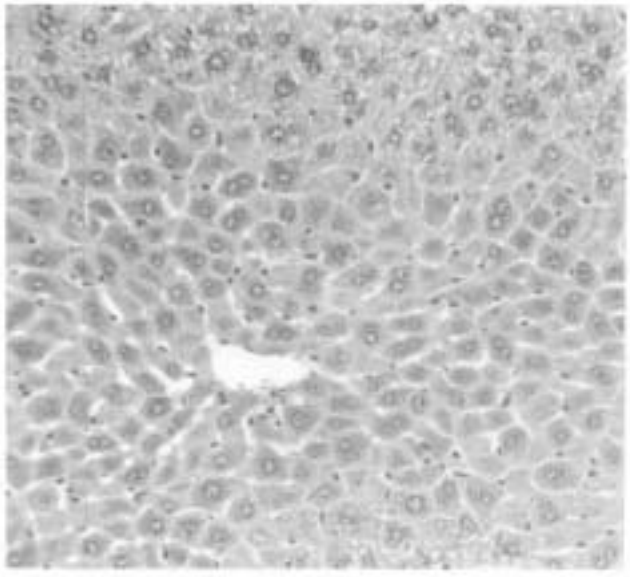


PLATE 2
Liver from a male mouse treated with 400 mg/kg AZT + 3,000 mg/kg pyrazinamide showing moderate glycogen depletion. (x160)

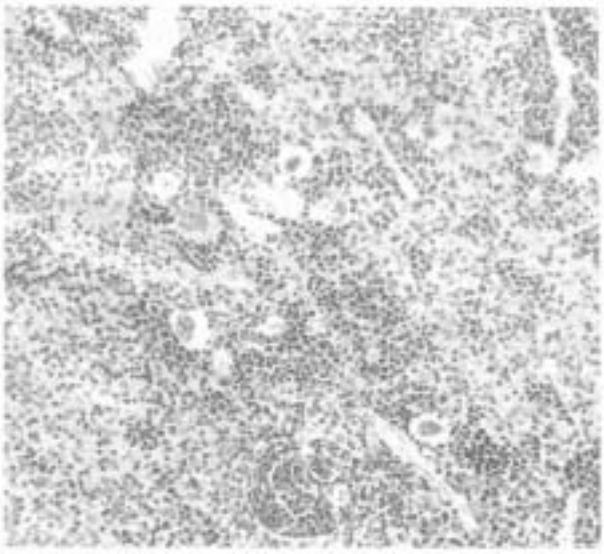


PLATE 3
Spleen from a control male mouse showing essentially normal hematopoietic cells. (x160)

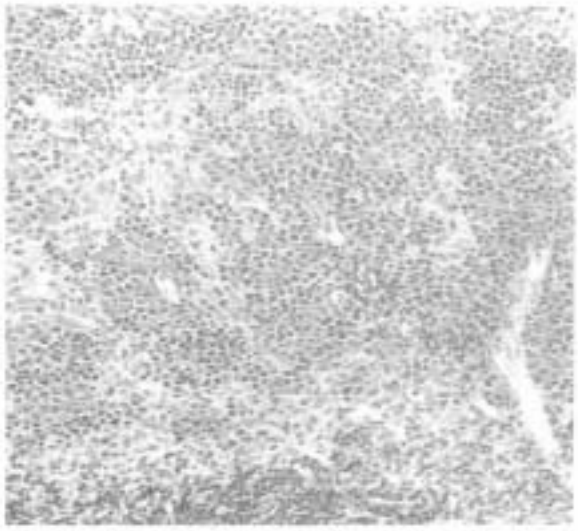


PLATE 4
Spleen from a male mouse treated with 400 mg/kg AZT + 3,000 mg/kg pyrazinamide showing moderate hematopoietic cell proliferation. (x160)

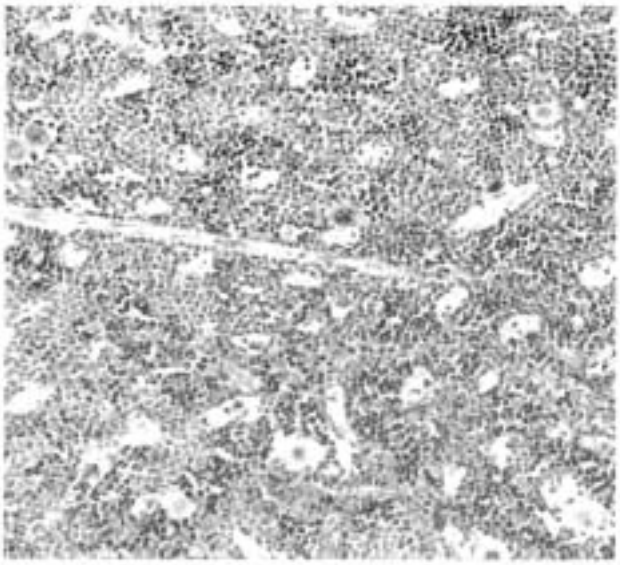


PLATE 5
Bone marrow from a control male mouse showing essentially normal hematopoietic elements. (x160)

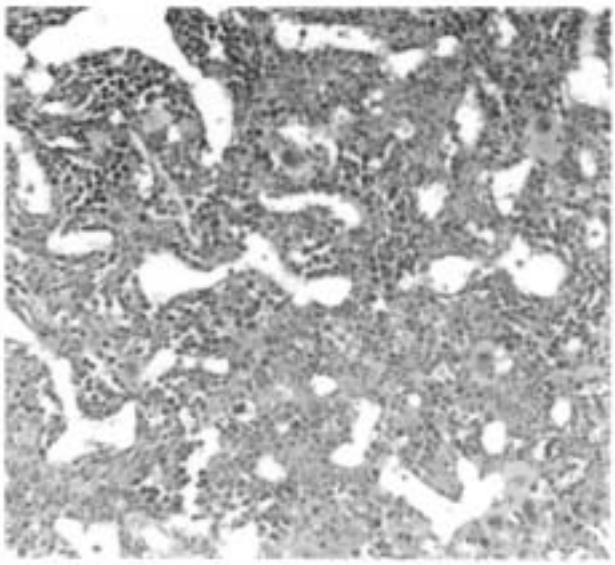


PLATE 6
Bone marrow from a male mouse treated with 400 mg/kg AZT + 3,000 mg/kg pyrazinamide showing mild cellular depletion. (x160)

Liver Lesions

Administration of AZT and pyrazinamide resulted in an increased incidence of a cytoplasmic alteration in hepatocytes diagnosed as glycogen depletion (Table 6). As glycogen does not stain with routine hematoxylin and eosin stains, this diagnosis represents a cytoplasmic alteration in hepatocytes manifested by diminished vacuolization. Criteria for severity grades of glycogen depletion of hepatocytes were:

> *Minimal severity* - a slight reduction of the irregularly shaped cytoplasmic vacuoles that are characteristic of the presence of glycogen in hepatocytes in areas surrounding the central veins, such areas usually extending not more than 1/3 to 1/2 the distance through the liver lobule from the central to portal veins
>
> *Mild severity* - an almost complete reduction of the irregularly shaped cytoplasmic vacuoles that are characteristic of the presence of glycogen in hepatocytes in areas surrounding the central veins, such areas usually extending not more than 1/3 to 1/2 the distance through the liver lobule from the central to portal veins
>
> *Moderate severity* - an almost complete reduction of the irregularly shaped cytoplasmic vacuoles that are characteristic of the presence of glycogen in hepatocytes in areas surrounding the central veins, such areas usually extending 1/2 the distance or more through the liver lobule from the central to portal veins
>
> *Marked severity* - an absence of the irregularly shaped cytoplasmic vacuoles that are characteristic of the presence of glycogen in hepatocytes.

Administration of AZT alone to male and female-A mice did not result in statistically significant increases ($P>0.05$) in the severity of glycogen depletion (Table 7). Compared to the vehicle control group, male and female-A mice treated with 1,500 or 3,000 mg/kg pyrazinamide alone had increased scores ($P\leq0.01$) for glycogen depletion. Compared to the male groups treated with 200 or 400 mg/kg AZT alone, the male mice that received 200 or 400 mg/kg AZT + 300 mg/kg pyrazinamide had significant ($P\leq0.05$) increases in the severity of glycogen depletion. A similar significant ($P\leq0.05$) increase in severity occurred in the female-A group treated with 400 mg/kg AZT + 300 mg/kg pyrazinamide, when compared to the female group treated with 400 mg/kg AZT alone. Compared to male and female-A groups treated with 200 or 400 mg/kg AZT alone, groups treated with AZT + 1,500 or 3,000 mg/kg pyrazinamide had even more significant ($P\leq0.01$) increases in severity of glycogen depletion. The group with the highest incidence and greatest severity grade was the female-A group treated with 400 mg/kg AZT + 3,000 mg/kg pyrazinamide. The severity score for this group was significant ($P\leq0.01$) when compared to the female-A group treated with 3,000 mg/kg pyrazinamide alone, indicating that the addition of 400 mg/kg AZT enhanced the severity of glycogen depletion. In general, the degree of severity of glycogen depletion correlated with increased relative liver weights. Necrosis of hepatocytes did not occur and a discernable increase in the size of the cells was not evident.

TABLE 6
Incidence and Severity of Hepatocellular Glycogen Depletion in Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

	Males		Females-A	
Dose[a]	Group Incidence[b]	Mean Severity[c]	Group Incidence[b]	Mean Severity[c]
0 + 0	2/10	3.0	6/20	2.5
200 + 0	2/10	2.5	11/20	2.8
400 + 0	6/10	2.2	8/20	2.6
0 + 300	6/10	2.5	11/20	2.2
0 + 1,500	10/10	2.9	20/20	3.1
0 + 3,000	9/10	3.2	20/20	3.1
200 + 300	8/10	2.5	16/20	2.4
200 + 1,500	10/10	3.3	20/20	3.0
200 + 3,000	9/10	3.3	17/20	3.2
400 + 300	8/10	3.0	17/20	2.9
400 + 1,500	10/10	3.2	20/20	3.0
400 + 3,000	9/10	3.6	20/20	3.8

[a] AZT + pyrazinamide in mg/kg per day

[b] Number of animals in group with lesion/number of animals with tissue examined microscopically.

[c] Mean severity for mice with the lesion (grade: 1=minimal, 2=mild, 3=moderate, and 4=marked)

TABLE 7
Statistical Analysis of Severity of Hepatocellular Glycogen Depletion in Swiss CD-l® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

	Severity [b] in Male Mice-			Severity [b] in Female-A Mice		
Dose[a]	Mean[c]	S.E.	Ratio [d]	Mean[c]	S.E.	Ratio
0+0	0.600	0.427	-	0.750	0.280	-
200+0	0.500	0.342	83	1.550	0.336	207
400+0	1.300	0.367	217	1.050	0-312	140
	Trend [e]	+ (P=0 109)		Trend [e]	+ (P=0.492)	
	Test Used [f]	Dunn		Test Used [f]	Dunn	
0+0	0.600	0.427	-	0.750	0.280	-
0+300	1.500	0.428	250	1.200	0.258	160
0+1,500	**2.900	0.100	483	**3.050	0.088	407
0+3,000	**2-900	0-379	483	**3.050	0.153	407
	Trend [e]	+ (P≤0.001)		Trend [e]	+ (P≤0.001)	
	Test Used [f]	Shirley		Test Used [f]	Shirley	
200+0	0.500	0.342	-	1.550	0.336	-
200+300	*2. 000	0.365	400	1.950	0256	126
200+1,500	**3.300	0.153	660	**3.000	0.126	194
200+3,000	**3.000	0.365	600	**2.700	0.300	174
	Trend [e]	+ (P≤0.001)		Trend [e]	+ (P≤0.001)	
	Test Used [f]	Shirley		Test Used [f]	Shirley	
400+0	1.300	0.367	-	1.050	0.312	
400+300	*2.400	0.427	185	**2.500	0.286	238
400+1,500	**3.200	0.200	246	**3.000	0.126	286
400+3,000	**3.200	0-389	246	**3.750	0.099	357
	Trend [e]	+ (P≤0.001)		Trend [e]	+ (P≤0.001)	
	Test Used [f]	Shirley		Test Used [f]	Shirley	
0+3,000	2.900	0.379	-	3.050	0.153	
200+3,000	3.000	0.365	103	2.700	0.300	89
400+3,000	3.200	0.389	110	**3.750	0.099	123
	Trend [e]	-(P≤0.001)		Trend [e]	- (P≤0.001)	
	Test Used [f]	Shirley		Test Used [f]	Shirley	

[a] AZT + pyrazinamide in mg/kg per day

[b] Severity grade is presented as mean severity for all mice in group, including those with no lesion (0=normal, 1 =minimal, 2=mild, 3=moderate, and 4=marked), and standard error (S.E.)

[c] n=l 0 for male groups and 20 for female-A groups

[d] Dosed group mean/control group mean) x 100

[e] Direction and significance of trend (Jonckheere's test)

[f] Multiple comparisons test comparing dose group to control group

* Significantly different from the control group (P≤0.05)

** Significantly different from the control group (P≤0.01)

Bone Marrow Lesions

Cellular depletion (depletion of hematopoietic cells) of bone marrow occurred only in male groups treated with 200 mg/kg AZT + 3,000 mg/kg pyrazinamide and 400 mg/kg AZT + 1,500 or 3,000 mg/kg pyrazinamide (Table 8). Criteria for severity of bone marrow lesions were as follows:

Minimal severity - Depletion of approximately 5% or less of the hematopoietic cells of the bone marrow, based on examination of the metaphyseal region

Mild severity - Depletion of approximately 6% to 20% of the hematopoietic cells of the bone marrow, based on examination of the metaphyseal region

Moderate severity - Depletion of approximately 21% to 50% of the hematopoietic cells of the bone marrow, based on examination of the metaphyseal region

Marked severity - Depletion of more than 50% of the hematopoietic cells of the bone marrow, based on examination of the metaphyseal region.

Compared to the male group treated with AZT alone, the severity was statistically significant ($P \leq 0.05$ to $P \leq 0.01$) in all three treatment groups (Table 9) and peaked in the group treated with the highest dose combination of AZT and pyrazinamide. In general, the degree of severity of bone marrow depletion corresponded with lower erythrocyte counts in the peripheral circulation.

TABLE 8
Incidence and Severity of Cellular Depletion of Bone Marrow in Male Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Dose[a]	Group Incidence[b]	Mean Severity[c]
0 + 0	0/10	-
200 + 3,000	4/10	1.8
400 + 1,500	3/10	1.3
400 + 3,000	5/10	2.0

Note: The lesion did not occur in dose groups not listed.

[a] AZT + pyrazinamide in mg/kg per day

[b] Number of animals in group with lesion/number of animals with tissue examined microscopically.

[c] Mean severity for mice with the lesion (grade: 1=minimal, 2=mild, 3=moderate, and 4=marked)

TABLE 9
Statistical Analysis of Mean Severity of Cellular Depletion of Bone Marrow in Male Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Dose[a] (Combination Groups Only)	Severity[b]	
	Mean[c]	S.E.
200 + 0	0.000	0.000
200 + 300	0.000	0.000
200 + 1,500	0.000	0.000
200 + 3,000	**0.700	0.300
	Trend[d]	+ (P=0.005)
	Test Used[e]	Shirley
400 + 0	0.000	0.000
400 + 300	0.000	0.000
400 + 1,500	*0.400	0.221
400 + 3,000	**1.000	0.365
	Trend[d]	+ (P=0.001)
	Test Used[e]	Shirley

[a] AZT + pyrazinamide in mg/kg per day

[b] Severity grade is presented as mean severity for all mice in group, including those with no lesion (0=normal, 1=minimal, 2=mild, 3=moderate, and 4=marked), and standard error (S.E.)

[c] n=10

[d] Direction and significance of trend (Jonckheere=s test)

[e] Multiple comparisons test comparing dose group to control group

* Significantly different from the control group ($P \leq 0.05$)

** Significantly different from the control group ($P \leq 0.01$)

Spleen Lesions

Hematopoietic cell proliferation of the spleen occurred in all male groups treated with AZT alone, pyrazinamide alone, or combinations of AZT and pyrazinamide (Table 10). Criteria for severity grades for hematopoietic cell proliferation were as follows:

Minimal severity - Approximately 15% or less of the red pulp is occupied by hematopoietic cells

Mild severity - Approximately 16% to 50% of the red pulp is occupied by hematopoietic cells

Moderate severity - Approximately 51% to 90% of the red pulp is occupied by hematopoietic cells

Marked severity - Approximately 91% to 100% of the red pulp is occupied by hematopoietic cells

Compared to the vehicle control group, the degree of severity was significant ($P \leq 0.01$) in the group treated with 400 mg AZT alone (Table 11). The degree of severity was not statistically significant ($P > 0.05$) in the male groups treated with pyrazinamide alone (compared to the vehicle controls) or combinations of AZT and pyrazinamide (compared to the group treated with AZT alone), indicating that this cellular response was primarily induced by AZT. Splenic hematopoiesis corresponded, in general, with declines in peripheral erythrocyte counts.

TABLE 10
Incidence and Severity of Spleen Hematopoiesis in Male Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Dose[a]	Group Incidence[b]	Mean Severity[c]
0 + 0	1/10	2.0
200 + 0	4/10	2.5
400 + 0	7/10	2.4
0 + 300	1/10	2.0
0 + 1,500	4/10	2.0
0 + 3,000	3/10	2.3
200 + 300	5/10	2.2
200 + 1,500	6/10	2.3
200 + 3,000	2/10	3.0
400 + 300	7/10	2.0
400 + 1,500	8/10	2.1
400 + 3,000	5/10	2.4

[a] AZT + pyrazinamide in mg/kg per day

[b] Number of animals in group with lesion/number of animals with tissue examined microscopically.

[c] Mean severity for mice with the lesion (grade: 1=minimal, 2=mild, 3=moderate, and 4=marked)

TABLE 11
Statistical Analysis of Severity of Splenic Hematopoiesis in Male Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

	Severity[b]		
Dose[a]	**Mean[c]**	**S.E.**	**Ratio[d]**
0 + 0	0.200	0.200	–
200 + 0	1.000	0.447	500
400 + 0	**1.700	0.396	850
	Trend[e]	+ (P=0.006)	
	Test Used[f]	Shirley	
0 + 0	0.200	0.200	–
0 + 300	0.200	0.200	100
0 + 1,500	0.800	0.327	400
0 + 3,000	0.700	0.367	350
	Trend[e]	+ (P=0.110)	
	Test Used[f]	Shirley	
200 + 0	1.000	0.447	–
200 + 300	1.100	0.379	110
200 + 1,500	1.400	0.400	140
200 + 3,000	0.600	0.427	60
	Trend[e]	- (P=0.604)	
	Test Used[f]	Shirley	
400 + 0	1.700	0.396	–
400 + 300	1.400	0.306	82
400 + 1,500	1.700	0.300	100
400 + 3,000	1.200	0.442	71
	Trend[e]	- (P=0.488)	
	Test Used[f]	Shirley	

a AZT + pyrazinamide in mg/kg per day
b Severity grade is presented as mean severity for all mice in group, including those with no lesion (0=normal, 1=minimal, 2=mild, 3=moderate, and 4=marked), and standard error (S.E.)
c n=10
d (Dosed group mean/control group mean) x 100
e Direction and significance of trend (Jonckheere's test)
f Multiple comparisons test comparing dose group to control group
* Significantly different from the control group (P≤0.05)
** Significantly different from the control group (P≤0.01)

Atrophy of the splenic red pulp occurred in three male mice each in the groups treated with 200 or 400 mg AZT + 3,000 mg pyrazinamide (Table 12). Splenic red pulp atrophy was graded for severity based on the following criteria:

Minimal severity – depletion of approximately 5% or less of normal red pulp cell population (erythroid cells, myeloid cells, megakaryocytes)

Mild severity - depletion of approximately 6 to 20% of normal red pulp cell population

Moderate severity - depletion of approximately 21 to 50% of normal red pulp cell population

Marked severity – depletion of 51% or greater of normal red pulp cell population.

The splenic atrophy that occurred in these mice likely contributed to a lowered incidence of splenic hematopoiesis in these two treatment groups. Compared to the group treated with AZT alone, the severity was significant ($P \leq 0.05$) in both treatment groups (Table 13) and corresponded with the hematological finding of anemia.

TABLE 12
Incidence and Severity of Red Pulp Atrophy of the Spleen in Male Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Dose[a]	Group Incidence[b]	Mean Severity[c]
0 + 0	0/10	B
200 + 3,000	3/10	2.0
400 + 3,000	3/10	2.3

Note: The lesion did not occur in dose groups not listed.

[a] AZT + pyrazinamide in mg/kg per day

[b] Number of animals in group with lesion/number of animals with tissue examined microscopically.

[c] Mean severity for mice with the lesion (grade: 1=minimal, 2=mild, 3=moderate, and 4=marked)

TABLE 13
Statistical Analysis of Severity of Red Pulp Atrophy in the Spleen of Male Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Dose[a] (Combination Groups Only)	Severity[b]	
	Mean[c]	S.E.
200 + 0	0.000	0.000
200 + 300	0.000	0.000
200 + 1,500	0.000	0.000
200 + 3,000	*0.600	0.306
	Trend[d] + (P=0.017)	
	Test Used[e] Dunn	
400 + 0	0.000	0.000
400 + 300	0.000	0.000
400 + 1,500	0.000	0.221
400 + 3,000	*0.700	0.367
	Trend[d] + (P=0.0171)	
	Test Used[e] Dunn	

[a] AZT + pyrazinamide in mg/kg per day
[b] Severity grade is presented as mean severity for all mice in group, including those with no lesion (0=normal, 1=minimal, 2=mild, 3=moderate, and 4=marked), and standard error (S.E.)
[c] n=10
[d] Direction and significance of trend (Jonckheere's test)
[e] Multiple comparisons test comparing dose group to control group
* Significantly different from the control group (P≤0.05)
** Significantly different from the control group (P≤0.01)

Other Lesions

Necrosis of various lymphoid tissues (spleen, lymph nodes, thymus) was seen in several male mice, most of which either died or underwent moribund sacrifice. All of these mice had received 3,000 mg/kg pyrazinamide either alone or in combination with AZT. The lymphoid tissue necrosis was considered a nonspecific stress-induced change rather than a direct drug-related lesion.

Lesions involving the bone, which was not required to be routinely examined microscopically, occurred in several male mice. The bone lesions usually appeared at necropsy as nodules or focal enlargements on the ventral aspect of the thoracic vertebrae or on the ribs near their junction with the thoracic vertebrae. Microscopically, these lesions appeared usually as hyperostosis or cartilaginous metaplasia with occasional associated fibrosis or necrosis. Although these changes were not seen in any control group mice, there was no evidence of a treatment-related pattern, and these lesions were believed to be secondary to the gavage procedure. Other lesions, such as hemorrhage, necrosis, and inflammation, occurred in a variety of tissues in a random pattern and were not believed to be associated with the test articles.

SPERM FUNCTION EVALUATION

Four of the male parameters were affected by treatment (Table 14). Administration of 200 or 400 mg/kg AZT alone resulted in a dose-related increase in left caudal weights that was statistically ($P \leq 0.05$) significant at the 200 mg/kg level. Mild elevations in caudal weight were not believed to be biologically significant.

A statistically significant decrease ($P \leq 0.01$) in epididymal sperm motility also occurred in the group treated with 200 mg/kg AZT. This finding was not considered to be biologically significant, as a dose-related pattern was not evident.

A decrease in mean left testicular weight occurred in the group treated with 3,000 mg/kg pyrazinamide alone. The mean left testicular weight was approximately 12% (0.1031 grams; $P \leq 0.01$) lower than the mean (0.1257 grams) in the control group.

For spermatid heads per gram of testis, there was a significant interaction (increase in counts) of both AZT and pyrazinamide ($P \leq 0.05$), indicating that the dose response relationship of AZT differs across doses of pyrazinamide. This interaction was not considered to have a negative impact on reproductive capacity. No other interaction effects occurred.

Table 14
Summary of Reproductive Tissue Evaluations in Male Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Dose[a]	Left Caudal Weight (g)	Left Testicular Weight (g)	Epididymal Sperm Motility (%)	Spermatid Heads Per Gram of Testis • 10^{7} [b]
0 + 0	0.0177 ± 0.0008	0.1257 ± 0.0049	79.57 ± 2.40 (9)	15.58 ± 0.44
200 + 0	0.0196 ± 0.0008[c]	0.1214 ± 0.0052	58.38 ± 6.65 (9)[e]	16.67 ± 0.70
400 + 0	0.0202 ± 0.0013	0.1280 ± 0.0044	74.20 ± 5.80 (9)	14.60 ± 0.44
0 + 300	0.0180 ± 0.0007	0.1227 ± 0.0039	74.45 ± 4.75	15.95 ± 0.42
0 + 1,500	0.0184 ± 0.0011	0.1206 ± 0.0066	72.16 ± 4.16	15.11 ± 0.64
0 + 3,000	0.0182 ± 0.0008 (9)	0.1031 ± 0.0026 (9)[d]	69.54 ± 6.53 (8)	15.18 ± 0.38 (9)
200 + 300	0.0197 ± 0.0011[c]	0.1293 ± 0.0047	54.10 ± 8.11[e]	15.55 ± 0.76
200 + 1,500	0.0192 ± 0.0007[c]	0.1188 ± 0.0042	70.68 ± 2.40 (9)[e]	15.23 ± 0.46
200 + 3,000	0.0225 ± 0.0039 (8)[c]	0.1090 ± 0.0039 (8)[d]	58.64 ± 8.86 (7)[e]	16.88 ± 0.49 (8)
400 + 300	0.0182 ± 0.0009	0.1155 ± 0.0041	68.58 ± 5.12 (9)	15.72 ± 0.31
400 + 1,500	0.0195 ± 0.0011	0.1181 ± 0.0062	64.57 ± 5.82 (9)	15.91 ± 0.49
400 + 3,000	0.0212 ± 0.0008 (8)	0.1137 ± 0.0056 (8)[d]	57.78 ± 7.94 (8)	16.86 ± 0.67 (8)

Note: All findings presented as mean value ± standard error; n=10 unless otherwise noted.
[a] AZT + pyrazinamide in mg/kg per day
[b] Significant interaction of both AZT and pyrazinamide ($P \leq 0.05$) indicating the dose response relationship of AZT differs across doses of pyrazinamide
[c] $P \leq 0.05$ compared to 0 + 0 (control) group by Williams' or Dunnett's test when collapsed over levels of pyrazinamide exposure
[d] $P \leq 0.01$ compared to 0 + 0 (control) group when collapsed over levels of AZT exposure
[e] $P \leq 0.01$ compared to 0 + 0 (control) group when collapsed over levels of pyrazinamide exposure

NATURAL DELIVERY DATA

The administration of AZT, pyrazinamide, or any combination of AZT and pyrazinamide did not affect the incidence of pregnancy (determined by the presence of a vaginal plug) in female-B mice (Table 15). Administration of 400 mg/kg AZT + 3,000 mg/kg pyrazinamide resulted in a marked decline ($P \leq 0.01$) in the number of pregnant female-B mice that delivered litters (Table 15). Of the mice that were pregnant in this group, 26.7% (4/15) delivered litters. The low number of delivered litters was not associated with inadequate implantation sites (Table 16).

Administration of 3,000 mg/kg pyrazinamide alone or in combination with AZT resulted in a slight increase in the duration of gestation (Table 16). Duration of gestation for the control group averaged 19.9 days. Respective mean durations of gestation for female-B mice treated with 3,000 mg/kg pyrazinamide, 200 mg/kg AZT + 3,000 mg/kg of pyrazinamide, and 400 mg/kg AZT + 3,000 mg/kg pyrazinamide were approximately 20.6 ($P \leq 0.01$), 20.5 ($P \leq 0.05$), and 20.5 days.

TABLE 15

Occurrence of Pregnancy in Female-B Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Dose[a]	Number Assigned	Number (%) Pregnant	Number (%) Delivered
0 + 0	15	15 (100)	15 (100)
200 + 0	15	14 (93.3)	14 (100)
400 + 0	15	14 (93.3)	14 (100)
0 + 300	16	14 (87.5)	13 (92.8)
0 + 1,500	16	14 (87.5)	13 (92.8)
0 + 3,000	16	14 (87.5)	13 (92.8)
200 + 300	16	12 (75.0)	11 (91.7)
200 + 1,500	16	16 (100)	16 (100)
200 + 3,000	16	15 (93.8)	13 (86.7)
400 + 300	16	13 (81.2)	13 (100)
400 + 1,500	16	13 (81.2)	13 (100)
400 + 3,000	16	15 (93.8)	4 (26.7)**

[a] AZT + pyrazinamide in mg/kg per day

** $P \leq 0.01$ compared to 0 + 0 (control) group by the Cochran-Armitage and Fisher's exact tests

TABLE 16
Summary of Natural Delivery Litter Data for Female-B Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Dose[a]	Litter Size (Mean ± SD[b])	Implantation Sites (Mean ± SD[b])	Duration of Gestation (Mean ± SD[b])	Dams with Stillborn Pups (% of Dams that Delivered)
0 + 0	12.5 ± 1.2	13.3 ± 1.0	19.9 ± 0.2	1 (6.7)
200 + 0	12.8 ± 1.2	13.6 ± 0.9	19.8 ± 0.4	0 (0.0)
400 + 0	10.5 ± 3.3	11.6 ± 3.7	20.1 ± 0.5	2 (14.3)
0 + 300	11.6 ± 1.7	12.4 ± 1.2	19.9 ± 0.3	0 (0.0)
0 + 1,500	11.4 ± 2.8	12.7 ± 2.9	20.0 ± 0.4	1 (7.7)
0 + 3,000	10.0 ± 3.5	11.3 ± 3.3	20.6 ± 0.6**	1 (7.7)
200 + 300	12.4 ± 2.2	13.7 ± 1.6	20.2 ± 0.4	0 (0.0)
200 + 1,500	11.3 ± 2.0	12.4 ± 1.0+	20.1 ± 0.3	2 (12.5)
200 + 3,000	9.2 ± 4.1*+	13.6 ± 1.4	20.5 ± 0.5*+	5 (38.5)+
400 + 300	10.7 ± 2.0	11.5 ± 1.7*	20.0 ± 0.0	0 (0.0)
400 + 1,500	8.9 ± 3.9*	12.2 ± 2.2	20.3 ± 0.6	3 (23.1)
400 + 3,000	8.7 ± 4.9	14.0 ± 0.8	20.5 ± 0.6	2 (50.0)

Dosea	Mean Live Litter Size (Mean ± SD[b])	Pups Dying on Days 1-4/Total Alive on Day 1 (%)	Cumulative Survival (Live Litter Size on Day 4/Day 0)	Live Litter Size at Weighing (Day 4) (Mean ± SD[b])
0 + 0	12.3 ± 1.1	0/185 (0.0)	12.3/12.3	12.3 ± 1.1
200 + 0	12.8 ± 1.2	1/164 (0.6)	12.5/12.8	12.5 ± 1.0
400 + 0	10.1 ± 3.6	10/139 (7.2)**	9.2/10.1	9.2 ± 3.8
0 + 300	11.6 ± 1.7	2/135 (1.5)	11.1/11.6	11.1 ± 2.0
0 + 1,500	11.2 ± 2.9	6/145 (4.1)*	10.7/11.2	10.7 ± 3.0
0 + 3,000	9.7 ± 3.9	1/101 (1.0)	9.1/9.7	9.1 ± 3.8
200 + 300	12.3 ± 2.3	4/135 (3.0)	11.9/12.3	11.9 ± 2.1
200 + 1,500	11.1 ± 2.1	6/178 (3.4)*	10.8/11.1	10.8 ± 2.5
200 + 3,000	8.5 ± 3.7**++	19/98 (19.4)**++	7.2/8.5**++	8.8 ± 2.3**++
400 + 300	10.6 ± 2.1	2/138 (1.4)-	10.5/10.6*	10.5 ± 2.0*
400 + 1,500	8.6 ± 4.2	10/112 (8.9)**	7.8/8.6**	7.8 ± 4.0**
400 + 3,000	6.7 ± 5.1	3/9 (33.3)**	2.0/6.7	6.0c

a AZT + pyrazinamide in mg/kg per day

b SD=standard deviation

c Value reported for only one dam (excludes values for one dam with no surviving pups and one dam with 11 pups found drowned on day 4).

* P≤0.05 compared to 0 + 0 (control) group by the Cochran-Armitage and Fisher=s exact tests (Dams with Stillborn Pups and Pups Dying on Days 1-4) or by Dunn=s test (all other parameters)

** P≤0.01 compared to 0 + 0 (control) group

\+ P≤0.05 compared to 200 + 0 group

++ P≤0.01 compared to 200 + 0 group

\- P≤0.05 compared to 400 + 0 group

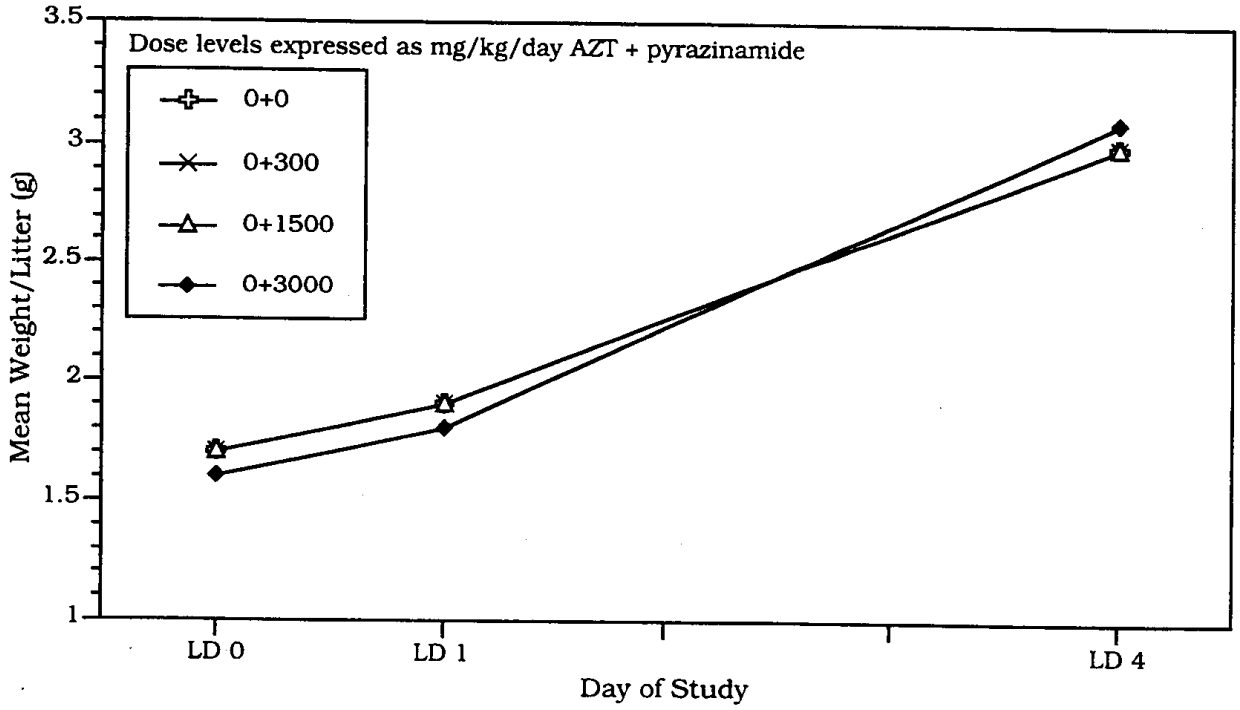
The percentage of dams with stillborn pups was increased in female-B groups treated with AZT in combination with the highest dose of pyrazinamide (Table 16). Compared to the group treated with 200 mg/kg AZT alone, which did not have stillborn pups, administration of 200 mg/kg AZT + 3,000 mg/kg pyrazinamide resulted in 5/13 (38.5%; $P \leq 0.05$) dams with stillborn pups. For the group treated with 400 mg/kg AZT + 3,000 mg/kg pyrazinamide, 2/4 (50%) dams had stillborn pups. This value was not statistically significant ($P > 0.05$) because of the low number of dams that delivered litters. None of the dams in any of the female-B treatment groups that delivered had all stillborn pups.

Treatment with higher combinations of AZT and pyrazinamide appeared to have a negative impact on litter size and live pups per litter (live litter size) on postnatal day 0. Compared to the control group, which averaged 12.5 pups per litter (Table 16), significant declines ($P \leq 0.05$) in litter size occurred in female-B groups treated with 200 mg/kg AZT + 3,000 mg/kg pyrazinamide or 400 mg/kg AZT + 1,500 mg/kg pyrazinamide. Respective mean litter sizes for these groups were 9.2 ($P \leq 0.05$) and 8.9 ($P \leq 0.05$) pups per litter. Although not statistically significant because of the low number of dams that delivered (4/15, 26.7%), the group treated with 400 mg/kg AZT + 3,000 mg/kg pyrazinamide had an average of 8.7 pups per litter on day 0. In general, the average number of liveborn pups per litter on day 0 (Table 16) paralleled the average litter size on day 0. Compared to the control group, which had 12.3 live pups per litter, the female-B group treated with 200 mg/kg AZT + 3,000 mg/kg pyrazinamide had 8.5 ($P \leq 0.01$) live pups per litter. Although not statistically significant ($P \geq 0.05$) when compared to the vehicle controls, the group treated with 400 mg/kg AZT + 3,000 mg/kg pyrazinamide averaged 6.7 pups per litter on day 0.

The percentage of pups dying on days 1 through 4 of the lactation period (Table 16) increased in a treatment-related pattern. For the group treated with 1,500 mg/kg pyrazinamide alone, 4.1% ($P \leq 0.05$) of the pups died. For the group treated with 400 mg/kg AZT alone, 7.2% ($P \leq 0.01$) of the pups died. Combination therapy resulted in an increase in the number of deaths in a dose-dependent pattern. For the female-B groups treated with 200 mg/kg AZT + 1,500 or 3,000 mg/kg pyrazinamide, 3.4% ($P \leq 0.05$) and 19.4% ($P \leq 0.01$) of the pups, respectively, died. For the groups treated with 400 mg/kg AZT + 1,500 or 3,000 mg/kg pyrazinamide, 8.9% ($P \leq 0.01$) and 33.3% ($P \leq 0.01$) of the pups died. None of the pups in the control group died.

Cumulative survival and live litter size at weighing (Table 16) declined in a treatment-related pattern. Although not statistically significant ($P > 0.05$) when compared to the control group, which had an average of 12.3 pups per litter on day 4 of lactation, the mean litter size in the female-B group treated with 400 mg/kg AZT alone was 9.2 pups per litter. Similar declines occurred in groups treated with 1,500 or 3,000 mg/kg pyrazinamide alone, these groups having 10.7 and 9.1 pups per litter, respectively. Combination therapy exaggerated the declines in litter size on day 4. For the female-B group treated with 200 mg/kg AZT + 3,000 mg/kg pyrazinamide, the mean litter size on day 4 was 7.2 ($P \leq 0.01$) pups per litter. For the groups treated with 400 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide, respective means for live litter size on day 4 were 10.5 ($P \leq 0.05$), 7.8 ($P \leq 0.01$), and 2.0 pups/litter. Similar treatment-related declines occurred in live litter size at the time of weighing on day 4.

Administration of 400 mg/kg AZT alone and 3,000 mg/kg pyrazinamide alone caused slight declines in pup body weight per litter on lactation day 0. Compared to litters in the control group, with pups weighing an average of 1.7 ± 0.1 grams, mean pup body weights for both of these two groups were 1.6 ± 0.2 grams. Further decreases in pup body weight occurred with combination therapy (Figure 11). For the group treated with 200 mg/kg AZT + 3,000 mg/kg pyrazinamide, the mean pup body weight on day 0 was approximately 18% (1.4 ± 0.2 grams; $P \leq 0.01$) lower than the mean (1.7 ± 0.1 grams) in the controls. For the groups treated with 400 mg/kg AZT + 1,500 or 3,000 mg/kg pyrazinamide, respective mean pup weights were approximately 12% (1.5 ± 0.1 grams; $P \leq 0.05$) and 30% (1.2 ± 0.2 grams; $P \leq 0.01$) lower than the mean (1.7 ± 0.1 grams) in the controls. These treatment-related trends continued during the lactation period on days 1 through 4. On lactation day 1, respective mean pup body weights in groups treated with 200 or 400 mg/kg AZT + 3,000 mg/kg pyrazinamide were approximately21.0% (1.5 ± 0.2 grams; $P \leq 0.01$) and 21.0% (1.5 ± 0.1 grams) lower than the mean (1.9 ± 0.1 grams) in the control group. On lactation day 4, respective mean pup body weights in groups treated with 200 or 400 mg/kg AZT + 3,000 mg/kg pyrazinamide were approximately 26.6% (2.2 ± 0.3 grams) and 33.3% (2.0 ± 0.0 grams) lower than the mean (3.0 ± 0.3 grams) in the control group.



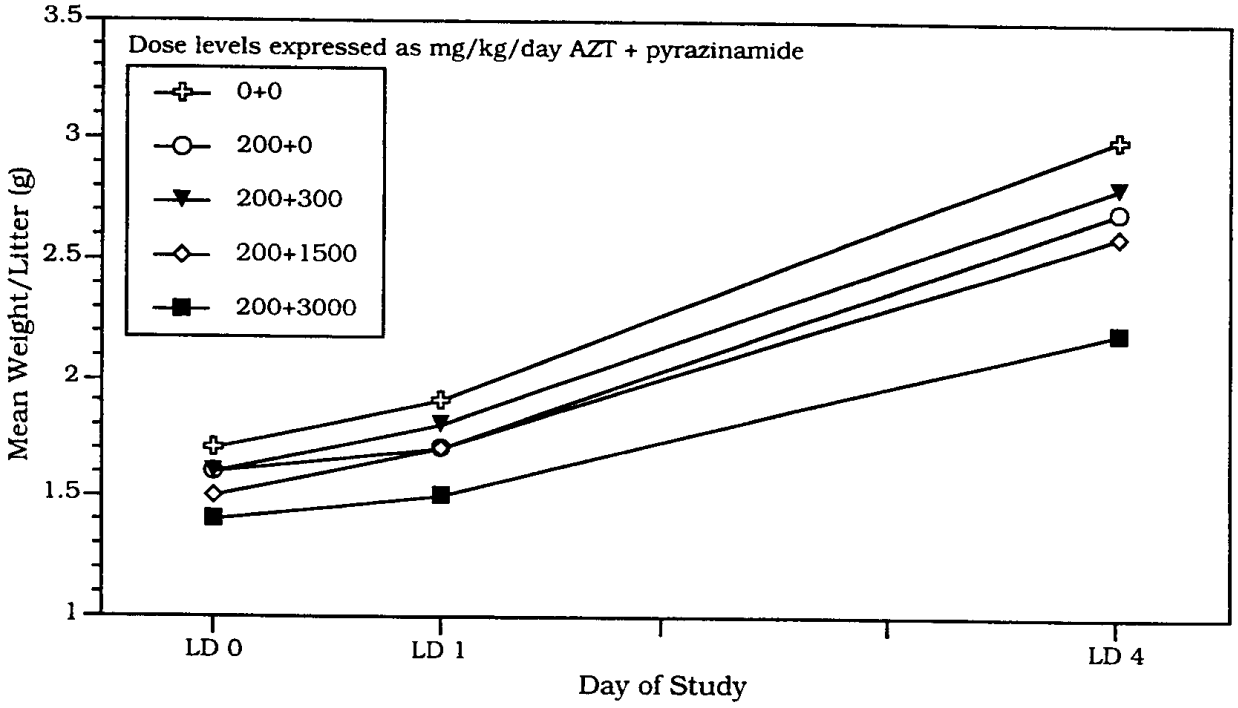


FIGURE 11
Mean Body Weights per Litter for Pups of Female-B Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations
(LD = Lactation Day; mean pup weight/litter include only values for litters in which pups survived to LD 4)

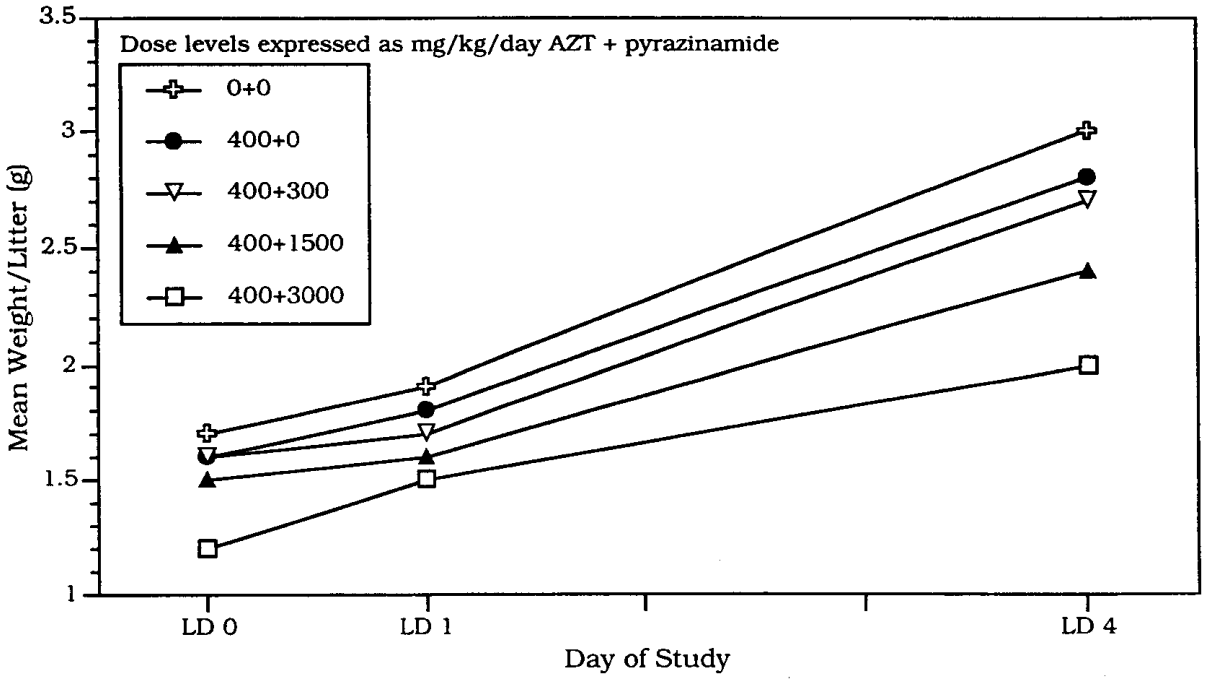


FIGURE 11 (continued)
Mean Body Weights per Litter for Pups of Female-B Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations
(LD = Lactation Day; mean pup weight/litter include only values for litters in which pups survived to LD 4)

CAESAREAN SECTION DATA

Administration of AZT alone (200 or 400 mg/kg) and pyrazinamide alone (300, 1,500, or 3,000 mg/kg) had no impact on the pregnancy rate of female-A mice (Table 17). Combination therapy resulted in a dose-related decline in the incidence of pregnancy. The pregnancy rate (percent of dams plugged) was 30% (6/20; P≤0.01) in the female-A group treated with 200 mg/kg AZT + 3,000 mg/kg pyrazinamide. For the female-A groups treated with 400 mg/kg AZT + 1,500 or 3,000 mg/kg pyrazinamide, respective pregnancy rates were 35% (7/20; P≤0.01) and 20% (4/20; P≤0.01). In general, the number of female-A mice caesarean-sectioned in each treatment group paralleled the number pregnant.

TABLE 17
Occurrence of Pregnancy in Female-A Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Dose[a]	Number Pregnant (%)[b]	Number Caesarean-Sectioned[c]
0 + 0	16 (80)	14
200 + 0	19 (95)	18
400 + 0	18 (90)	18
0 + 300	17 (85)	16
0 + 1,500	18 (90)	17
0 + 3,000	18 (90)	17
200 + 300	15 (75)	15
200 + 1,500	14 (70)+	14
200 + 3,000	6 (30)**++	5
400 + 300	19 (95)	17
400 + 1,500	7 (35)**-	7
400 + 3,000	4 (20)**-	4

a AZT + pyrazinamide in mg/kg per day
b n=20. Pregnancy was determined by the presence of a vaginal plug.
c Values were excluded for dams that delivered, died, or were Caesarean-sectioned prior to scheduled sacrifice.
** P≤0.01 compared to 0 + 0 (control) group by the Cochran-Armitage and Fisher=s exact tests
+ P≤0.05 compared to 200 + 0 group
++ P≤0.01 compared to 200 + 0 group
- P≤0.01 compared to 400 + 0 group

Litter size and the number of live fetuses per litter were not altered by pyrazinamide alone (Table 18). Administration of AZT alone caused a reduction in live litter size. Compared to the control group, with an average of 10.9 live fetuses per litter, female-A groups treated with 200 or 400 mg/kg AZT alone had 8.3 and 3.9 (P≤0.01) live fetuses per litter, respectively. Live litter size was also reduced in female-A groups treated with higher combinations of AZT and pyrazinamide. The group treated with 200 mg/kg AZT + 3,000 mg/kg pyrazinamide

TABLE 18
Summary of Caesarean-Sectioning Litter Data for Female-A Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

	Corpora		Litter Size	Live Fetuses		Resorptions	
Dose[a]	Lutea	Implantation s	Mean ± SD[b]	n	Mean ± SD[b]	n	Mean ± SD[b]
0 + 0	12.1 ± 3.3	11.4 ± 3.2	10.9 ± 3.0	153	10.9 ± 3.0	6	0.4 ± 0.5
200 + 0	13.0 ± 2.7	11.3 ± 3.4	8.4 ± 4.0	150	8.3 ± 4.0	52	2.9 ± 2.7**
400 + 0	12.5 ± 3.1	9.3 ± 9.3	3.9 ± 3.4**	71	3.9 ± 3.4**	96	5.3 ± 3.9**
0 + 300	11.1 ± 3.9	10.6 ± 3.5	9.8 ± 4.3	157	9.8 ± 4.3	12	0.8 ± 1.5
0 + 1,500	12.0 ± 4.2	10.6 ± 3.5	9.4 ± 3.3	146	8.6 ± 3.9	21	1.2 ± 1.3
0 + 3,000	14.0 ± 2.3	13.2 ± 2.4	11.6 ± 2.6	188	11.0 ± 2.5	28	1.6 ± 1.1*
200 + 300	12.3 ± 3.4	9.8 ± 4.1	7.7 ± 4.0	114	7.6 ± 4.0	32	2.1 ± 1.3**
200 + 1,500	12.4 ± 3.3	11.6 ± 3.0	8.6 ± 3.9	118	8.4 ± 3.8	42	3.0 ± 2.5*
200 + 3,000	9.0 ± 5.1	5.8 ± 2.8	1.0 ± 1.4*+	5	1.0 ± 1.4*+	24	4.8 ± 1.6**
400 + 300	11.7 ± 3.1	10.5 ± 3.5	4.6 ± 4.6**	78	4.6 ± 4.6**	100	5.9 ± 4.4**
400 + 1,500	11.6 ± 3.2	9.8 ± 4.2	4.7 ± 3.5	33	4.7 ± 3.5*	36	5.1 ± 3.1**

[a] AZT + pyrazinamide in mg/kg per day
[b] Standard deviation
* P≤0.05 compared to 0 + 0 (control) group by Dunn=s test
** P≤0.01 compared to 0 + 0 (control) group
\+ P≤0.05 compared to 200 + 0 group

averaged 1 (P≤0.05) live fetus per litter. For the female-A groups treated with 400 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide, the average numbers of live fetuses per litter, respectively, were 4.6 (P≤0.01), 4.7 (P≤0.05), and 1.8 (P≤0.05).

No significant (P<0.05) differences were noted between the number of corpora lutae or implantations per litter (Table 18) when treated female-A groups were compared to untreated controls.

An increase in the mean number of resorptions (Table 18) occurred in female-A groups treated with AZT alone, pyrazinamide alone, and combinations of AZT and pyrazinamide. For the female-A groups treated with 200 or 400 mg/kg AZT alone, the mean numbers of resorptions were 2.9 (P≤0.01) and 5.3 (P≤0.01) per dam, respectively. For the group treated with 3,000 mg/kg pyrazinamide alone, 1.6 (P≤0.05) resorptions per dam occurred. In general, the number of resorptions in groups treated with combination therapy increased in a dose-dependent pattern. For the female-A groups treated with 200 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide, the mean numbers of resorptions were 2.1 (P≤0.01), 3.0 (P≤0.01), and 4.8 (P≤0.01), respectively. For groups treated

with 400 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide, 5.9 (P≤0.01), 5.1 (P≤0.01), and 10.2 (P≤0.05) resorptions per dam, respectively, occurred.

Treatment-related declines in average fetal weight per litter (Table 19) occurred in female-A groups receiving AZT alone, pyrazinamide alone, and combinations of AZT and pyrazinamide. Respective mean fetal weight per litter for groups treated with 200 or 400 mg/kg AZT alone were approximately 14% (1.25 grams; P≤0.01) and 21% (1.14 grams; P≤0.01) lower than the mean (1.45 grams) in the control group. For the group treated with 3,000 mg/kg pyrazinamide alone, the mean fetal weight per litter was approximately 30% (1.01 grams; P≤0.01) lower than the control-group mean (1.45 grams). Further declines with a dose-dependent pattern occurred with combination therapy. For the female-A groups receiving 200 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide, respective mean fetal weights per litter were approximately 17% (1.21 grams; P≤0.01), 23% (1.11 grams; P≤0.01) and 44% (0.81 grams) lower than the mean (1.45 grams) in the control group. For the groups treated with 400 mg/kg AZT + 300, 1,500, or 3,000 mg/kg pyrazinamide, respective mean fetal weights per litter were approximately 24% (1.10 grams; P≤0.01), 32% (0.99 grams; P≤0.01), and 48% (0.76 grams) lower than the mean (1.45 grams) in the control group.

TABLE 19
Body Weights of Fetuses from Female-A Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Dose[a]	Mean Fetal Weight (g) Mean ± SD[b]
0 + 0	1.45 ± 0.16
200 + 0	1.25 ± 0.13**
400 + 0	1.14 ± 0.18*
0 + 300	1.47 ± 0.14
0 + 1,500	1.34 ± 0.10
0 + 3,000	1.01 ± 0.07**
200 + 300	1.21 ± 0.19**
200 + 1,500	1.11 ± 0.14**
200 + 3,000	0.81 ± 0.10
400 + 300	1.10 ± 0.05**
400 + 1,500	0.99 ± 0.12**
400 + 3,000	0.76 ± 0.25

a AZT + pyrazinamide in mg/kg per day
b Standard deviation
* P≤0.05 compared to 0 + 0 (control) group by Scheffe's or Dunn's test
** P≤0.01 compared to 0 + 0 (control) group

GROSS EXTERNAL ALTERATIONS (FEMALE-A LITTERS)

Administration of AZT alone, pyrazinamide alone, or combinations of AZT and pyrazinamide did not result in significant (P≤0.05) increases in the fetal or litter incidence of external abnormalities (Table 20). Although the frequency of external alterations was greater in groups treated with pyrazinamide alone, none of the abnormalities occurred in a dose-dependent pattern and none were statistically significant (P>0.05) when compared to the control group.

TABLE 20
Summary of Gross Alterations in Fetuses from Female-A Swiss CD-1® Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Observation	Dose[a]	Litter Incidence[b]	Fetal Incidence[c]
Head, exencephaly	0 + 1,500	2 (13.3)	2 (1.4)
	0 + 3,000	3 (17.6)	3 (1.6)
	400 + 300	1 (9.1)	1 (1.3)
Head, encephalocele	0 + 3,000	1 (5.9)	1 (0.5)
Head, hematoma	0 + 1,500	1 (6.7)	1 (0.7)
Eyes, open	0 + 3,000	1 (5.9)	1 (0.5)
Ears, low set	0 + 3,000	1 (5.9)	1 (0.5)
Palate, cleft	0 + 3,000	1 (5.9)	1 (0.5)
	200 + 1,500	2 (15.4)	2 (1.7)
Tongue, protrudes	0 + 3,000	1 (5.9)	1 (0.5)
Body, edema	400 + 3,000	1 (50.0)	1 (14.3)
Limbs, clubfoot	0 + 0	3 (21.4)	6 (3.9)
	0 + 300	1 (6.2)	1 (0.6)
	0 + 1,500	1 (6.7)	2 (1.4)
	0 + 3,000	1 (5.9)	1 (0.5)
	200 + 0	2 (11.8)	2 (1.3)
Tail, hematoma	0 + 0	1 (7.1)	1 (0.6)

[a] AZT + pyrazinamide in mg/kg per day

[b] Number of litters with the observation followed by the percent (number of litters with the observation/number of litters evaluated).

[c] Number of fetuses with the observation followed by the percent (number of fetuses with the observation/number of fetuses evaluated; excludes dead fetuses).

DISCUSSION AND CONCLUSIONS

A reproductive, developmental, and general toxicity study was conducted in Swiss CD-1® mice treated with AZT alone (200 or 400 mg/kg per day), pyrazinamide alone (300, 1,500, or 3,000 mg/kg per day), or combinations of AZT and pyrazinamide. The test articles were administered by oral gavage for approximately 20 days to male mice, approximately 30 days to female mice (female-A), and approximately 10 days to female mice (female-B).

Measurable evidence of hematopoietic toxicity occurred in male and female mice treated with AZT alone. The most significant alteration in male and female-A mice treated with AZT alone consisted of a mild anemia accompanied by elevated MCV and MCH values. The anemia was related to dose and duration of treatment as the severity was greatest in female-A mice treated for approximately 30 days when compared to the male mice treated for approximately 20 days. Hematological alterations in female-B mice treated with AZT alone for approximately 10 days were limited to mild elevations in MCV values. In general, the mild macrocytic anemia that occurred in male mice was accompanied by a dose-related increase in the incidence of splenic hematopoiesis. The mild macrocytic anemia that occurred with AZT treatment is compatible with the anemia previously reported in mice (Thompson *et al.*, 1991). Macrocytic anemia is a common observation in humans treated with AZT (Snower and Weil, 1993; Richman *et al.*, 1987). The exact mechanism of the erythrocyte macrocytosis is unclear but it likely reflects inhibition of DNA synthesis in erythroid precursors. AZT is directly toxic to erythroid blast forming units (BFU-E) and erythroid colony forming units (CFU-E) *in vitro* at high concentrations and is antiproliferative in CFU-E at lower concentrations (Gogu *et al.*, 1995). In *in vivo* studies, AZT has been shown to increase splenic and bone marrow BFU-E in mice and to increase the sensitivity of both splenic and bone marrow BFU-E to erythropoietin (Chow *et al.*, 1991); both effects occur in the absence of an appreciable regenerative response (reticulocytosis), suggesting a maturation block in the erythroid series due to a block in terminal differentiation. AZT has since been shown to down-regulate the erythropoietin receptor in CFU-E and inhibit erythropoietin receptor-mediated signal transduction (Gogu *et al.*, 1995). These mechanisms could inhibit CFU-E proliferation and possibly affect erythropoietin-regulated maturation (Bick, 1993). The absence of reticulocytosis in the presence of a macrocytic anemia subsequent to the administration of AZT in this study is compatible with depression of the erythroid precursors due to impaired DNA synthesis.

Measurable evidence of liver toxicity occurred in mice of both sexes treated with pyrazinamide alone. Male and female-A mice treated with pyrazinamide alone had increased incidences and severity of hepatocullular glycogen depletion accompanied by increased absolute and relative liver weights. Elevated serum enzyme levels did not occur. The hepatocellular glycogen depletion and elevated liver weights that occurred with pyrazinamide treatment are compatible with those previously described (Rao *et al.*, 1998; NIEHS, 2000) in B6C3F1 mice treated with 1,000 or 1,500 mg/kg pyrazinamide. The manifestations of liver toxicity in mice in this study are different from those described in human patients treated with pyrazinamide. Pyrazinamide-induced liver toxicity in human patients (Mandel and Sande, 1990; Durand *et al.*, 1996) is manifested by elevated alanine and aspartate aminotransferase levels and liver necrosis. Liver necrosis and increased liver enzyme levels did not occur in male or female mice in this study.

Administration of AZT and pyrazinamide in combination to male and female mice resulted in exacerbation of the anemia produced by AZT alone. Combination therapy also resulted in thrombocytosis, leukopenia, neutropenia, and lymphopenia. In addition to the splenic hematopoiesis previously described with AZT treatment alone, male mice receiving combination therapy also had a treatment-related depletion of bone marrow. Exacerbation of hematopoietic toxicity subsequent to combination therapy may be the result of pyrazinamide interfering with AZT metabolism. The liver is considered the major site for AZT catabolism and some AZT catabolites are 5- to 7-fold more toxic than AZT (Cretton *et al.*, 1991).

Administration of AZT alone, pyrazinamide alone, or combinations of AZT and pyrazinamide did not result in significant compound-related alterations in mortality, clinical chemistry parameters, or necropsy findings in mice of either sex. Significant alterations in body weight and body weight gains were not evident in any of the treated male groups. Biologically significant declines in body weight were detected in female mice. Terminal body weights and gravid uterine weights were diminished in female-A mice treated with AZT alone or AZT + pyrazinamide. Corrected body weights for these treatment groups were similar to controls. A slight decline in gestational body weight was evident in the female-B group treated with 400 mg/kg AZT + 3,000 mg/kg pyrazinamide. Significant clinical signs were limited to a few pale animals in the female-A group treated with the highest combination of both test articles. Although occasional statistically significant alterations occurred in epididymal sperm motility, caudal weights, testicular weights, and spermatid heads per gram of testis, none of the sperm function parameters were considered to reflect diminished reproductive capacity.

Measurable evidence of reproductive toxicity was evident in groups treated with AZT alone, pyrazinamide alone, and combinations of AZT and pyrazinamide. Administration of AZT alone resulted in diminished litter size, an increase in the numbers of resorptions, and diminished fetal weights per litter. These alterations have been previously reported in mice treated with AZT alone (NIEHS, 1999). Treatment with pyrazinamide alone resulted in a mild increase in the number of resorptions, diminished fetal weights per litter, and a mild increase in the duration of gestation. In a previous study with lower dosages of pyrazinamide (NIEHS, 1997), significant alterations in reproductive parameters did not occur. Combination therapy resulted in alterations in reproductive parameters of far greater magnitude than detected subsequent to the administration of either test article alone. Combination therapy resulted in a decrease in the number of pregnant females that delivered litters, diminished litter size, an increase in the number of dams with stillborn pups, and a decrease in the number of liveborn pups per litter. During the lactation period, combination therapy resulted in diminished live litter size and an increase in the number of pup deaths. For the mice that were caesarean-sectioned, combination therapy resulted in a diminished number of pregnancies, reduced live litter size, an increase in the number of resorptions, and a decline in fetal weights per litter. Reproductive toxicity with combination therapy may be a reflection of the severity of maternal toxicity, because the reduction in litter size for female-A dams treated for approximately 30 days was far greater than in female-B dams treated for approximately 10 days. Significant numbers of gross external alterations in fetuses did not occur following treatment with AZT alone, pyrazinamide alone, or combinations of AZT and pyrazinamide.

Combinations of AZT at 200 or 400 mg/kg with pyrazinamide at 3,000 mg/kg increased liver weights, increased the hematopoietic toxicity as indicated by anemia, and may have increased the mortality compared to mice treated with the corresponding doses of either drug alone. These effects indicate maternal toxicity at the high dose combinations. Because most reproductive and developmental toxic effects in female mice were observed at the high dose combinations that caused maternal toxicity, increases in reproductive and developmental toxicity at these doses may be secondary to increased maternal toxicity. Pyrazinamide alone up to 10 times the therapeutic dose did not cause biologically significant reproductive, developmental, or general toxicity, which suggests a high safety factor for therapy with pyrazinamide. At 20 times the therapeutic dose, pyrazinamide alone caused a slight increase in liver weights and a significant decrease in mean fetal weight with no significant effects on reproductive parameters.

CONCLUSIONS

Administration of AZT alone (200 or 400 mg/kg per day) to male and female Swiss CD-1® mice caused

hematopoietic toxicity manifested primarily by a mild macrocytic anemia. Administration of pyrazinamide alone (300, 1,500, or 3,000 mg/kg per day) resulted in liver toxicity manifested by increased liver weights and hepatocellular glycogen depletion. Pyrazinamide administered in combination with AZT exacerbated the hematopoietic toxicity of AZT.

Regarding reproductive toxicity, administration of AZT alone resulted in an increase in the number of resorptions, diminished litter size, and diminished fetal weights per litter. Pyrazinamide alone resulted in a mild increase in the number of resorptions, diminished fetal body weights per litter, and a mild increase in the duration of gestation. Combination therapy resulted in alterations in the above reproductive parameters of far greater magnitude than those resulting from either compound alone. Other changes evident subsequent to combination therapy consisted of a decrease in the number of pregnant females that delivered litters, diminished litter size, an increase in the number of stillborn pups, and a decrease in the number of liveborn pups per litter. Combination therapy also resulted in diminished live litter size and an increase in the number of pup deaths during the lactation period. Significant numbers of gross external alterations did not occur in fetuses following administration of either compound alone or in combination.

Pyrazinamide alone up to 10 times the therapeutic dose did not cause biologically significant reproductive, developmental, or general toxicity, indicating a high safety factor for therapy with pyrazinamide. Even at 20 times the therapeutic dose, pyrazinamide caused only minimal toxicity. However, results of this study indicate that the toxicological effects of combination therapy could be more severe than the toxicity of individual therapies, even though one of the therapies, such as pyrazinamide, may have minimal toxicity.

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APPENDIX A
BODY WEIGHTS AND ORGAN WEIGHTS

TABLE A1
Group Mean Body Weights of Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Male Mice

Dose[a]	Mean Body Weights[b] on Study Day						
	3	5	9	13	17	21	23
0 + 0	37.9 ± 1.8	38.2 ± 1.5	37.6 ± 1.4	37.0 ± 1.6	37.8 ± 2.0	37.1 ± 1.8	38.0 ± 1.9
200 + 0	38.1 ± 1.3	38.2 ± 1.7	37.6 ± 1.7	37.1 ± 1.7	37.9 ± 1.8	37.8 ± 1.8	37.9 ± 1.9
400 + 0	37.3 ± 1.9	37.3 ± 1.6	37.4 ± 1.3	36.7 ± 1.2	37.4 ± 1.6	37.3 ± 1.2	37.9 ± 1.8
0 + 300	37.2 ± 1.7	36.4 ± 3.6	37.6 ± 1.8	37.2 ± 2.1	37.6 ± 2.2	37.4 ± 2.4	37.8 ± 2.5
0 + 1,500	37.7 ± 2.2	38.9 ± 2.6	38.6 ± 2.1	38.3 ± 2.3	38.8 ± 2.2	39.0 ± 2.3	39.6 ± 2.2
0 + 3,000	37.1 ± 2.3	37.7 ± 2.4	36.9 ± 2.2 (9)	37.6 ± 2.6 (9)	38.9 ± 2.5 (9)	39.7 ± 2.8 (9)	39.7 ± 2.8 (9)
200 + 300	37.6 ± 2.2	37.4 ± 2.4	37.7 ± 2.0	37.3 ± 1.7	37.7 ± 1.7	37.6 ± 1.5	37.9 ± 1.5
200 + 1,500	37.1 ± 1.5	37.1 ± 1.8	37.7 ± 1.6	36.9 ± 2.1	37.5 ± 2.2	37.8 ± 2.3	38.8 ± 2.2
200 + 3,000	37.4 ± 1.8	37.7 ± 2.9	37.2 ± 2.2 (8)	37.4 ± 1.3 (8)	38.1 ± 1.6 (8)	38.8 ± 1.6 (8)	38.6 ± 1.6 (8)
400 + 300	37.4 ± 1.8	36.5 ± 2.6	37.5 ± 1.8	36.8 ± 1.4	38.2 ± 1.5	36.9 ± 3.2	38.0 ± 1.8
400 + 1,500	36.6 ± 2.3	37.5 ± 1.9	38.6 ± 2.0	37.7 ± 2.4	38.8 ± 2.8	38.5 ± 2.4	38.4 ± 2.4
400 + 3,000	38.2 ± 1.8	38.8 ± 2.0	39.2 ± 1.8 (8)	39.1 ± 1.7 (8)	40.5 ± 2.3 (8)	39.9 ± 2.0 (8)	40.2 ± 2.6 (8)

[a] Doses expressed as AZT + pyrazinamide in mg/kg per day

[b] Weights (grams) expressed as group mean ± standard deviation; n=10 unless otherwise noted in parentheses

TABLE A1
Group Mean Body Weights of Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Female-A Mice

Dose[a]	Mean Body Weights[b] on Study Day						
	3	5	9	13	17	21	23
0 + 0	28.3 ± 1.4	28.9 ± 1.4	29.3 ± 1.5	31.4 ± 2.2	33.2 ± 2.8	37.2 ± 4.9	43.2 ± 8.2
200 + 0	29.6 ± 2.0	30.4 ± 1.7	29.9 ± 2.0	31.6 ± 2.1	33.6 ± 3.0	37.8 ± 4.3	43.6 ± 6.3 (19)
400 + 0	29.4 ± 1.7	29.6 ± 1.7	29.6 ± 2.5	30.4 ± 1.7	32.4 ± 1.7	34.8 ± 2.6	36.7 ± 4.7
0 + 300	29.3 ± 2.1	29.5 ± 2.1	29.8 ± 1.7	31.2 ± 2.0	33.2 ± 2.3	37.4 ± 4.3	43.5 ± 7.0
0 + 1,500	29.3 ± 2.0	29.6 ± 1.8	30.1 ± 1.4	32.2 ± 1.7	34.8 ± 2.4	38.8 ± 3.9	44.6 ± 6.3
0 + 3,000	29.4 ± 2.0	29.8 ± 1.6	30.3 ± 1.8	33.0 ± 2.0	35.4 ± 3.1	39.6 ± 4.1 (19)	44.6 ± 6.0
200 + 300	29.4 ± 1.7	29.4 ± 1.6	29.6 ± 1.7	31.2 ± 1.8	32.8 ± 2.3	35.5 ± 4.1	39.0 ± 6.8
200 + 1,500	29.8 ± 1.2	30.3 ± 1.1	30.4 ± 1.5	32.8 ± 1.6	34.8 ± 2.5	37.2 ± 4.8	39.9 ± 7.4
200 + 3,000	30.2 ± 1.6	31.0 ± 1.5	30.7 ± 1.7 (18)	32.6 ± 1.6 (18)	34.4 ± 2.0 (17)	34.6 ± 3.1 (17)	34.4 ± 2.5*++ (17)
400 + 300	29.2 ± 1.4	29.7 ± 1.4	29.5 ± 1.4	30.4 ± 2.3	32.6 ± 2.3 (19)	35.7 ± 3.5 (19)	38.3 ± 6.4 (18)
400 + 1,500	29.7 ± 1.3	30.4 ± 1.2	30.4 ± 1.3	31.7 ± 1.5	32.8 ± 1.9	33.4 ± 2.6	33.6 ± 4.1**
400 + 3,000	30.4 ± 2.2	31.6 ± 2.2	31.8 ± 2.1*	33.4 ± 1.7–	34.0 ± 2.3	34.2 ± 2.7	33.8 ± 3.5*

a Doses expressed as AZT + pyrazinamide in mg/kg per day
b Weights (grams) expressed as group mean ± standard deviation; n=20 unless otherwise noted in parentheses
* $P \leq 0.05$ compared to 0 + 0 (control) group by Scheffe's or Dunn's test
** $P \leq 0.01$ compared to 0 + 0 (control) group by Scheffe's or Dunn's test
++ $P \leq 0.01$ compared to 200 + 0 group by Scheffe's or Dunn's test
– $P \leq 0.01$ compared to 400 + 0 group by Scheffe's or Dunn's test

TABLE A1
Group Mean Body Weights of Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Female-B Mice

		Mean Body Weights[b] on Study Day						
		Day of Gestation				Day of Lactation		
Dose[a]	n	0	8	12	15	0	1	4
0 + 0	15	29.7 ± 1.3	34.4 ± 2.0	41.8 ± 4.0	49.8 ± 3.1	37.1 ± 2.1	38.0 ± 2.3	44.8 ± 3.0
200 + 0	14	29.4 ± 1.2	34.6 ± 1.7	40.8 ± 2.5	48.8 ± 3.2	36.3 ± 2.3	37.4 ± 1.8	44.0 ± 2.3
400 + 0	14	29.3 ± 1.1	34.3 ± 2.3	40.4 ± 3.5	46.6 ± 4.2	36.7 ± 2.5	36.2 ± 2.9	41.75.2
0 + 300	14	29.3 ± 1.6	34.1 ± 1.6	40.8 ± 2.6	48.3 ± 3.5	36.5 ± 2.1 (13)	37.3 ± 3.1 (13)	44.3 ± 3.2 (13)
0 + 1,500	14	29.7 ± 1.6	35.2 ± 2.2	41.4 ± 2.8	47.7 ± 4.0	36.0 ± 3.4 (13)	37.9 ± 3.0 (13)	44.0 ± 2.8 (13)
0 + 3,000	14	29.3 ± 1.4	35.1 ± 2.0	39.8 ± 3.2	46.0 ± 4.0	36.4 ± 2.7 (11)	37.4 ± 2.9 (11)	43.0 ± 4.1 (11)
200 + 300	12	29.2 ± 1.8	33.9 ± 2.0	38.6 ± 3.6	46.3 ± 3.8	35.6 ± 2.1 (11)	37.0 ± 2.2 (11)	42.4 ± 4.2 (11)
200 + 1,500	16	29.2 ± 1.3	34.2 ± 1.7	39.8 ± 2.2	46.1 ± 2.4	36.4 ± 1.6	36.5 ± 2.0	40.8 ± 4.1
200 + 3,000	15	29.7 ± 1.2	35.4 ± 2.3	40.7 ± 2.9	47.3 ± 2.5 (14)	37.0 ± 1.9 (12)	36.6 ± 1.4 (12)	41.6 ± 3.5 (12)
400 + 300	13	29.4 ± 1.3	34.1 ± 1.4	39.4 ± 1.7	46.5 ± 2.4	36.4 ± 2.0	36.5 ± 2.1	42.8 ± 2.9
400 + 1,500	13	29.8 ± 1.4	35.2 ± 2.8	40.8 ± 3.3	46.9 ± 3.9	37.1 ± 2.6	36.6 ± 2.6	40.4 ± 5.7
400 + 3,000	15	29.5 ± 1.2	34.9 ± 1.7	40.4 ± 2.2	43.7 ± 3.2*	37.7 ± 0.6 (3)	36.5 ± 0.7 (3)	39.0 ± 0.0 (3)

a Doses expressed as AZT + pyrazinamide in mg/kg per day
b Weights (grams) expressed as group mean ± standard deviation; n=20 unless otherwise noted in parentheses
* $P \leq 0.05$ compared to 0 + 0 (control) group by Scheffe's or Dunn's test
** $P \leq 0.01$ compared to 0 + 0 (control) group by Scheffe's or Dunn's test
++ $P \leq 0.01$ compared to 200 + 0 group by Scheffe's or Dunn's test
– $P \leq 0.01$ compared to 400 + 0 group by Scheffe's or Dunn's test

TABLE A2
Group Mean Organ Weights of Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Male Mice

	Body	Right Epididymis Weight[b]		Liver Weight[b]	
Dose[a]	Weight[b]	Absolute	Relative	Absolute	Relative
0 + 0	38.15	0.04552 ± 0.0645	1.2 ± 0.2	2.0830 ± 0.2734	54.6 ± 7.1
200 + 0	39.79	0.04534 ± 0.00529 (- 0.4)	1.1 ± 0.1 (- 4.6)	2.1510 ± 0.2615 (+ 3.3)	54.0 ± 4.0 (- 1.1)
400 + 0	38.60	0.04495 ± 0.00599 (- 1.3)	1.2 ± 0.2 (- 2.1)	2.0080 ± 0.2917 (- 3.6)	51.9 ± 6.0 (- 4.9)
0 + 300	38.50	0.04147 ± 0.00450 (- 8.9)	1.1 ± 0.2 (- 9.3)	2.2120 ± 0.3978 (+ 6.2)c	57.2 ± 6.8 (+ 4.7)
0 + 1,500	40.33*	0.04794 ± 0.00512 (+ 5.3)	1.2 ± 0.1 (- 0.6)	2.3780 ± 0.3048 (+ 14.2)*	58.8 ± 5.0 (+ 7.7)
0 + 3,000[c]	40.19*	0.04183 ± 0.00352 (- 8.1)	1.0 ± 0.1 (- 12.4)*	2.3567 ± 0.1667 (+ 13.1)*	58.7 ± 3.6 (+ 7.6)
200 + 300	39.40	0.04770 ± 0.00585 (+ 4.8)	1.2 ± 0.2 (+ 1.8)	2.1630 ± 0.2001 (+ 3.8)	54.8 ± 3.2 (+ 0.5)
200 + 1,500	39.54	0.04833 ± 0.00518 (+ 6.2)	1.2 ± 0.1 (+ 2.6)	2.3570 ± 0.3599 (+ 13.2)*	59.4 ± 6.6 (+ 8.9)*
200 + 3,000[d]	39.66	0.04574 ± 0.00546 (+ 0.5)	1.2 ± 0.1 (- 3.7)	2.5200 ± 0.2700 (+ 21.0)*	63.4 ± 4.9 (+ 16.2)*
400 + 300	38.79	0.04359 ± 0.00587 (- 4.2)	1.1 ± 0.2 (- 5.8)	2.1780 ± 0.2288 (+ 4.6)	56.1 ± 4.9 (+ 2.8)
400 + 1,500	39.15	0.04903 ± 0.00706 (+ 7.7)	1.3 ± 0.2 (+ 4.8)	2.3300 ± 0.2053 (+ 11.9)*	59.5 ± 3.7 (+ 9.0)*
400 + 3,000[d]	41.06*	0.04548 ± 0.00718 (- 0.1)	1.1 ± 0.2 (- 7.0)	2.5500 ± 0.2240 (+ 22.4)*	62.2 ± 4.9 (+ 13.8)*

a Doses expressed as AZT + pyrazinamide in mg/kg per day
b Values are presented as mean ± standard deviation (% difference from control); n=10 unless otherwise noted. Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.
c n=9
d n=8
* $P \leq 0.05$ compared to the 0 + 0 (control) group by the Student's t-test

TABLE A2
Group Mean Organ Weights of Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Female-A Mice

Dose[a]	n	Corrected Body Weight[b]	Liver Weight[c] Absolute	Relative
0 + 0	13	37.40	2.7069 ± 0.2643	72.5 ± 5.9
200 + 0	15	36.28	2.4387 ± 0.3601 (- 9.9)	66.9 ± 5.3 (- 7.6)*
400 + 0	16	36.01	2.2581 ± 0.3822 (- 16.6)*	62.4 ± 6.2 (- 13.9)*
0 + 300	15	37.33	2.7733 ± 0.3219 (+ 2.5)	74.5 ± 6.9 (+ 2.7)
0 + 1,500	12	37.96	2.9517 ± 0.3150 (+ 9.0)	77.6 ± 5.4 (+ 7.1)
0 + 3,000	13	39.29	3.2400 ± 0.3844 (+ 19.7)*	82.4 ± 7.4 (+ 13.8)*
200 + 300	14	37.04	2.6436 ± 0.4133 (- 2.3)	71.0 ± 7.1 (- 2.0)
200 + 1,500	12	38.08	2.9908 ± 0.5169 (+ 10.5)	78.1 ± 9.4 (+ 7.7)*
200 + 3,000	5	35.83	2.4900 ± 0.3195 (- 8.0)	69.4 ± 6.2 (- 4.3)
400 + 300	15	35.88	2.4140 ± 0.5276 (- 10.8)	66.6 ± 9.1 (- 8.2)
400 + 1,500	6	36.80	2.6733 ± 0.4301 (- 1.2)	72.4 ± 8.4 (- 0.1)
400 + 3,000	4	36.02	2.8525 ± 0.3024 (+ 5.4)	79.1 ± 2.3 (+ 9.1)

a Doses expressed as AZT + pyrazinamide in mg/kg per day
b Total body weight minus gravid uterine weight in grams
c Values are presented as mean ± standard deviation (% difference from control). Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.
n Number of values included in calculations. Values exclude early deaths, dams designated as not pregnant, dams that delivered or began delivery prior to scheduled sacrifice, and dams that were sacrificed on an estimated day 14 or 18 of gestation.
* $P \leq 0.05$ compared to the 0 + 0 (control) group by the Student's t-test

APPENDIX B
CLINICAL PATHOLOGY RESULTS

TABLE B1
Hematology Data for Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations[a]

Male Mice

	WBC 10^3/L	RBC 10^6/L	HGB g/dL	HCT %	MCV fL	MCH pg	PLT 10^3/L	RETIC 10^5/L	NEUT 10^3/L	LYMPH 10^3/L
0 + 0 mg/kg per day[b]										
Mean	6.19	9.37	15.3	45.1	48.2	16.4	932	5.0	1.00	4.77
SD	1.260	1.346	2.17	6.86	2.10	0.77	274.2	0.57	0.395	0.827
n	10	10	10	10	10	10	10	10	10	10
200 + 0 mg/kg per day										
Mean	6.29	8.22	14.3	43.5	53.2**	17.5	1,147	5.4	1.52	4.37
SD	3.121	1.062	1.80	4.41	3.86	0.89	290.7	1.39	1.772	1.642
n	10	10	10	10	10	10	10	10	10	10
400 + 0 mg/kg per day										
Mean	5.67	8.18	14.3	43.7	53.6**	17.5	1,129	6.5	5.67	8.18
SD	1.279	1.096	1.96	5.21	3.25	0.69	208.9	1.49	1.279	1.096
n	10	10	10	10	10	10	10	10	10	10
0 + 300 mg/kg per day										
Mean	6.52	9.98	16.5	48.4	48.7	16.5	878	5.4	1.08	5.05
SD	1.632	1.314	1.80	4.93	2.56	0.68	257.3	2.12	0.440	1.319
n	10	10	10	10	10	10	10	10	10	10
0 + 1,500 mg/kg per day										
Mean	7.35	9.73	16.0	47.1	48.5	16.4	1,059	5.2	1.61	5.24
SD	2.457	1.034	1.82	4.76	2.05	0.71	137.2	1.22	0.800	1.960
n	10	10	10	10	10	10	10	10	10	10
0 + 3,000 mg/kg per day										
Mean	5.19	9.49	15.8	46.8	49.3	16.7	1,163	5.1	1.18	3.69
SD	2.209	0.492	0.93	2.55	2.19	0.69	256.2	0.82	0.526	1.736
n	10	9	9	9	9	9	9	9	9	9
200 + 300 mg/kg per day										
Mean	5.96	8.47	14.5	43.4	51.5	17.3	1,247	5.0	0.77	4.81
SD	1.855	0.825	0.91	2.78	3.31	1.18	172.3	1.11	0.224	1.569
n	10	10	10	10	10	10	10	9	10	10
200 + 1,500 mg/kg per day										
Mean	5.21	8.14	14.3	43.2	53.2**	17.6	1,553**	5.2	1.18	3.62
SD	1.804	0.854	1.45	4.25	2.40	0.66	210.8	1.12	0.598	1.320
n	10	10	10	10	10	10	10	10	10	10
200 + 3,000 mg/kg per day										
Mean	4.93	5.78**	9.6**	27.7**	47.7	16.6	1,978**	3.3	0.81	3.72
SD	1.796	0.876	1.58	5.29	3.55	0.66	362.7	2.18	0.251	1.642
n	8	8	8	8	8	8	8	8	8	8

TABLE B1
Hematology Data for Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Male Mice

	WBC 10^3/L	RBC 10^6/L	HGB g/dL	HCT %	MCV fL	MCH pg	PLT 10^3/L	RETIC 10^5/L	NEUT 10^3/L	LYMPH 10^3/L
400 + 300 mg/kg per day										
Mean	5.04	8.27	14.5	43.4	52.6*	17.5	1,172	4.9	0.60	4.07
SD	1.875	1.097	1.98	5.58	1.95	0.69	202.1	0.84	0.270	1.528
n	10	10	10	10	10	10	10	10	10	10
400 + 1,500 mg/kg per day										
Mean	5.56	7.94*	13.9	41.9	52.9**	17.5	1,420**	5.0	1.19	3.92
SD	1.758	0.466	1.10	3.44	3.52	0.96	266.1	1.21	0.636	1.504
n	10	10	10	10	10	10	10	9	10	10
400 + 3,000 mg/kg per day										
Mean	3.58	5.71**	9.6**	27.9**	48.5	16.7	1,824**	3.0	0.77	2.44
SD	1.304	1.030	2.17	7.16	4.12	0.91	563.2	2.09	0.689	1.014
n	8	8	8	8	8	8	8	7	8	8

[a] WBC=leukocytes, RBC=erythrocytes, HGB=hemoglobin, HCT=hematocrit, MCV=mean cell volume, MCH=mean cell hemoglobin, PLT=platelets, RETIC=reticulocytes, NEUT=neutrophils, LYMPH=lymphocytes

[b] AZT + pyrazinamide

* Significantly different ($P<0.05$) from the control by Dunnett's test

** Significantly different ($P<0.01$) from the control by Dunnett's test

TABLE B1
Hematology Data for Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations[a]

Female-A Mice

	WBC 10^3/L	RBC 10^6/L	HGB g/dL	HCT %	MCV fL	MCH pg	PLT 10^3/L	RETIC 10^5/L	NEUT 10^3/L	LYMPH 10^3/L
0 + 0 mg/kg per day[b]										
Mean	6.85	9.14	15.1	45.0	49.3	16.5	1,190	4.3	1.83	4.61
SD	2.915	0.664	0.98	2.35	2.32	0.77	290.7	1.27	1.410	1.622
n	20	20	20	20	20	20	20	20	20	20
200 + 0 mg/kg per day										
Mean	5.95	7.88**	14.5	44.4	56.4**	18.4**	1,319	4.0	1.43	4.23
SD	1.311	0.644	1.12	3.56	3.09	0.86	248.4	1.13	0.642	0.881
n	20	20	20	20	20	20	20	20	20	20
400 + 0 mg/kg per day										
Mean	5.96	7.62**	14.4	44.4	58.3**	18.8**	1,378	4.7	1.29	4.23
SD	1.403	0.546	1.06	3.12	2.56	0.53	326.1	1.46	0.745	1.058
n	20	20	20	20	20	20	20	20	20	20
0 + 300 mg/kg per day										
Mean	6.95	8.80	14.9	44.2	50.3	17.0	1,251	4.6	1.91	4.65
SD	2.304	0.891	1.33	3.91	1.69	0.68	288.8	1.64	1.237	1.683
n	20	20	20	20	20	20	20	20	20	20
0 + 1,500 mg/kg per day										
Mean	6.73	9.08	15.5	45.7	50.5	17.1	1,347	4.8	1.98	4.31
SD	2.114	0.952	1.49	3.99	2.36	0.81	335.7	1.71	0.737	1.457
n	20	20	20	20	20	20	20	20	20	20
0 + 3,000 mg/kg per day										
Mean	7.07	9.33	16.3	48.4	52.0	17.4*	1,266	5.2	2.47	4.11
SD	1.704	0.657	1.08	3.02	2.67	0.77	308.8	1.64	1.216	0.923
n	20	20	20	20	20	20	20	20	20	20
200 + 300 mg/kg per day										
Mean	5.49	7.94**	14.8	44.4	56.1**	18.8**	1,174	4.3	1.28	3.93
SD	1.501	0.602	0.97	3.06	2.86	0.83	277.4	1.50	0.810	1.021
n	20	20	20	20	20	20	20	19	20	20
200 + 1,500 mg/kg per day										
Mean	5.25*	7.91**	15.0	46.1	58.5**	19.0**	1,262	4.3	1.16	3.74
SD	1.374	0.814	1.20	3.42	3.81	1.05	268.9	2.04	0.566	1.126
n	20	20	20	20	20	20	20	20	20	20
200 + 3,000 mg/kg per day										
Mean	4.28**	7.88**	15.1	46.2	58.2**	19.2**	1,277	4.9	0.98*	3.02
SD	1.118	1.329	2.71	8.53	4.21	0.92	421.7	2.35	0.704	0.610
n	17	17	17	17	17	17	17	17	17	17

TABLE B1
Hematology Data for Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Female-A Mice

	WBC 10^3/L	RBC 10^6/L	HGB g/dL	HCT %	MCV fL	MCH pg	PLT 10^3/L	RETIC 10^5/L	NEUT 10^3/L	LYMPH 10^3/L
400 + 300 mg/kg per day										
Mean	5.61	7.98**	14.9	45.4	57.0**	18.8**	1,291	4.5	0.99*	4.32
SD	1.398	0.872	1.46	4.08	2.48	0.66	327.6	1.64	0.409	1.193
n	19	19	19	19	19	19	19	19	19	19
400 + 1,500 mg/kg per day										
Mean	4.97**	7.82**	14.9	45.5	58.2**	19.1**	1,295	4.7	0.88**	3.74
SD	0.967	0.571	0.80	2.37	3.07	0.97	379.3	1.78	0.347	0.766
n	20	20	20	20	20	20	20	20	20	20
400 + 3,000 mg/kg per day										
Mean	4.21**	5.69**	10.4**	31.7**	53.6**	17.8**	2,166**	4.6	0.71**	3.23
SD	1.392	2.226	4.53	15.15	7.49	1.66	773.5	3.10	0.441	1.058
n	20	20	20	20	20	20	20	20	20	20

[a] WBC=leukocytes, RBC=erythrocytes, HGB=hemoglobin, HCT=hematocrit, MCV=mean cell volume, MCH=mean cell hemoglobin, PLT=platelets, RETIC=reticulocytes, NEUT=neutrophils, LYMPH=lymphocytes

[b] AZT + pyrazinamide

* Significantly different ($P<0.05$) from the control by Dunnett's test

** Significantly different ($P<0.01$) from the control by Dunnett's test

TABLE B1
Hematology Data for Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations[a]

Female-B Mice

	WBC	RBC	HGB	HCT	MCV	MCH	PLT	RETIC	NEUT	LYMPH
	10^3/L	10^6/L	g/dL	%	fL	pg	10^3/L	10^5/L	10^3/L	10^3/L
0 + 0 mg/kg per day[b]										
Mean	5.87	8.51	14.4	43.4	51.1	17.0	1,294	5.5	1.43	4.08
SD	1.877	0.504	0.96	2.12	1.78	0.56	252.4	1.78	0.812	1.245
n	15	15	15	15	15	15	15	15	15	15
200 + 0 mg/kg per day[b]										
Mean	5.78	8.15	14.1	43.2	53.2	17.4	1,278	6.1	1.35	4.06
SD	1.090	0.844	1.08	2.77	2.92	0.73	312.7	2.27	0.497	0.653
n	15	15	15	15	15	15	15	15	15	15
400 + 0 mg/kg per day[b]										
Mean	6.09	7.98	14.1	42.6	53.6*	17.7	1,277	5.2	1.11	4.53
SD	1.674	0.924	1.59	3.84	2.55	0.66	279.6	1.14	0.364	1.497
n	15	15	15	15	15	15	15	15	15	15
0 + 300 mg/kg per day										
Mean	6.43	8.68	14.6	44.2	51.0	16.8	1,289	5.3	1.22	4.88
SD	1.631	0.782	1.11	3.40	2.34	0.53	261.0	1.22	0.460	1.271
n	15	15	15	15	15	15	15	15	15	15
0 + 1,500 mg/kg per day										
Mean	5.78	9.02	15.4	46.0	51.1	17.1	1,371	4.7	1.11	4.37
SD	1.762	1.043	1.48	3.97	2.48	0.64	347.6	1.23	0.417	1.601
n	15	15	15	15	15	15	15	15	15	15
0 + 3,000 mg/kg per day										
Mean	5.88	9.29	15.6	46.6	50.3	16.8	1,289	4.2	1.22	4.23
SD	1.954	0.754	1.20	2.26	2.46	0.73	300.8	1.46	0.443	1.582
n	16	16	16	16	16	16	16	16	16	16
200 + 300 mg/kg per day										
Mean	6.44	8.56	14.9	45.1	52.8	17.4	1,345	6.1	1.28	4.77
SD	2.317	0.912	1.45	4.07	2.08	0.65	342.6	1.55	0.375	1.904
n	16	16	16	16	16	16	16	16	16	16
200 + 1,500 mg/kg per day										
Mean	6.56	8.15	14.9	45.2	55.5**	18.3**	1,484	6.0	1.46	4.69
SD	1.941	0.797	1.44	4.06	1.91	0.56	270.9	2.12	0.531	1.664
n	16	16	16	16	16	16	16	16	16	16
200 + 3,000 mg/kg per day										
Mean	6.21	8.36	15.1	45.5	54.6**	18.1**	1,376	4.8	1.46	4.33
SD	1.401	0.766	1.09	3.25	2.91	0.88	252.9	1.87	0.934	1.136
n	15	15	15	15	15	15	15	15	15	15

TABLE B1
Hematology Data for Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Female-B Mice

	WBC 10^3/L	RBC 10^6/L	HGB g/dL	HCT %	MCV fL	MCH pg	PLT 10^3/L	RETIC 10^5/L	NEUT 10^3/L	LYMPH 10^3/L
400 + 300 mg/kg per day										
Mean	6.61	8.14	14.3	44.0	54.3**	17.6	1,321	6.1	1.55	4.63
SD	1.898	0.648	0.74	2.20	2.44	0.82	203.5	1.53	0.976	1.070
n	16	16	16	16	16	16	16	16	16	16
400 + 1,500 mg/kg per day										
Mean	6.31	8.61	15.4	46.1	53.8*	17.9**	125	5.4	1.57	4.38
SD	2.690	1.263	1.70	5.02	2.74	0.89	274.8	1.52	1.358	1.646
n	16	16	16	16	16	16	16	16	16	16
400 + 3,000 mg/kg per day										
Mean	6.59	8.89	15.4	47.2	53.2	17.4	1,392	5.4	1.55	4.60
SD	2.319	0.880	1.17	3.36	2.54	0.83	288.6	1.99	1.418	1.908
n	16	16	16	16	16	16	16	16	16	16

[a] WBC=leukocytes, RBC=erythrocytes, HGB=hemoglobin, HCT=hematocrit, MCV=mean cell volume, MCH=mean cell hemoglobin, PLT=platelets, RETIC=reticulocytes, NEUT=neutrophils, LYMPH=lymphocytes

[b] AZT + pyrazinamide

* Significantly different ($P< 0.05$) from the control by Dunnett's test

** Significantly different ($P< 0.01$) from the control by Dunnett's test

TABLE B2
Clinical Chemistry Data for Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Male Mice

	Alkaline Phosphatase U/L	Alanine Aminotransferase U/L	Aspartate Aminotransferase U/L	Sorbitol Dehydrogenase U/L	Total Bile Acids µmol/L
0 + 0 mg/kg per day[a]					
Mean	56	29	60	25	9
SD	15.9	13.7	20.8	6.6	3.1
n	10	8	3	6	3
200 + 0 mg/kg per day					
Mean	56	27	89	19	22
SD	3.3	12.2	58.8	3.9	12.4
n	10	10	6	4	6
400 + 0 mg/kg per day					
Mean	58	27	56	25	7
SD	14.9	9.3	26.4	4.2	7.8
n	10	10	5	3	2
0 + 300 mg/kg per day					
Mean	61	26	51	10	5
SD	19.1	10.2	21.0	9.0	4.2
n	10	8	6	3	2
0 + 1,500 mg/kg per day					
Mean	55	42	66	26	24
SD	14.8	13.4	26.6	8.4	19.1
n	10	10	4	3	3
0 + 3,000 mg/kg per day					
Mean	49	27	40	24	16
SD	9.6	10.0	3.8	10.0	11.2
n	9	9	3	6	3
200 + 300 mg/kg per day					
Mean	55	34	63	26	24
SD	18.9	13.8	15.1	4.7	20.5
n	10	10	4	4	2
200 + 1,500 mg/kg per day					
Mean	55	30	68	34	21
SD	19.3	10.9	16.9	19.7	6.5
n	10	10	7	6	4
200 + 3,000 mg/kg per day					
Mean	59	40	58	37	23
SD	27.8	32.0	11.4	4.1	9.9
n	8	8	6	5	6

TABLE B2
Clinical Chemistry Data for Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Male Mice

	Alkaline Phosphatase U/L	Alanine Aminotransferase U/L	Aspartate Aminotransferase U/L	Sorbitol Dehydrogenase U/L	Total Bile Acids µmol/L
400 + 300 mg/kg per day					
Mean	57	28	60	19	20
SD	14.0	12.4	23.6	8.0	9.5
n	10	9	7	3	6
400 + 1,500 mg/kg per day					
Mean	55	32	46	34	23
SD	17.4	15.5	27.0	14.5	4.9
n	10	10	5	5	2
400 + 3,000 mg/kg per day					
Mean	49	65	54	38	25
SD	20.2	93.2	19.2	11.1	9.1
n	8	8	7	4	6

[a] AZT + pyrazinamide

TABLE B2Clinical Chemistry Data for Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Female-A Mice

	Alkaline Phosphatase U/L	Alanine Aminotransferase U/L	Aspartate Aminostransferase U/L	Sorbitol Dehydrogenase U/L	Total Bile Acids µmol/L
0 + 0 mg/kg per day[a]					
Mean	68	36	84	19	26
SD	21.5	15.5	40.5	6.6	20.4
n	20	20	16	18	15
200 + 0 mg/kg per day					
Mean	69	31	87	21	19
SD	15.8	11.2	31.3	8.9	8.1
n	20	19	17	17	14
400 + 0 mg/kg per day					
Mean	82	28	93	21	22
SD	17.8	10.4	36.3	7.4	12.1
n	20	20	12	19	12
0 + 300 mg/kg per day					
Mean	55	34	80	20	18
SD	16.7	9.3	21.2	9.6	6.9
n	20	20	14	17	10
0 + 1,500 mg/kg per day					
Mean	63	36	83	27	22
SD	17.6	10.0	20.5	8.9	11.1
n	20	19	14	16	10
0 + 3,000 mg/kg per day					
Mean	64	36	88	26	43
SD	16.7	13.3	26.7	11.6	26.1
n	20	20	13	16	7
200 + 300 mg/kg per day					
Mean	63	32	88	26	18
SD	17.1	9.1	27.3	10.3	7.8
n	20	19	16	15	12
200 + 1,500 mg/kg per day					
Mean	70	41	73	28	33
SD	19.4	26.6	30.2	8.8	22.0
n	20	20	16	19	13
200 + 3,000 mg/kg per day					
Mean	80	26	65	26	16
SD	55.5	9.5	29.2	6.9	6.1
n	17	14	10	11	6

TABLE B2
Clinical Chemistry Data for Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Pyrazinamide Combinations

Female-A Mice

	Alkaline Phosphatase U/L	Alanine Aminotransferase U/L	Aspartate Aminotransferase U/L	Sorbitol Dehydrogenase U/L	Total Bile Acids µmol/L
400 + 300 mg/kg per day					
Mean	72	31	88	25	20
SD	13.2	10.7	24.6	6.0	9.5
n	19	19	15	14	9
400 + 1,500 mg/kg per day					
Mean	69	33	70	29	24
SD	14.7	11.8	20.1	8.4	8.0
n	20	20	15	15	10
400 + 3,000 mg/kg per day					
Mean	71	28	64	33	26
SD	51.9	9.2	21.9	8.8	13.1
n	20	19	16	18	15

[a] AZT + pyrazinamide

Other NIEHS Reports and Publications on Toxicology of AIDS Therapeutics:

National Institute of Environmental Health Sciences (NIEHS) (1997). Reproductive, Developmental, and General Toxicity Studies of Pyrazinamide Administered by Gavage to Swiss (CD-1®) Mice. NIEHS AIDS Therapeutics Toxicity Report No. 1. NIH Publication No. 97-3938. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. Research Triangle Park, NC.

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Zhuang, S.-M., Eklund, L.K., Cochran, C., Rao, G.N., Wiseman, R.W., and Soderkvist, P. (1996). Allelotype analysis of 2',3'-dideoxycytidine- and 1,3-butadiene-induced lymphomas in B6C3F1 Mice. *Cancer Res.* **56**, 3338-3343.

The NIEHS reports may be accessed at the NIEHS AIDS World Wide Web site:
http://ntp-server.niehs.nih.gov/Main_Pages/AIDS/AIDSpage.html